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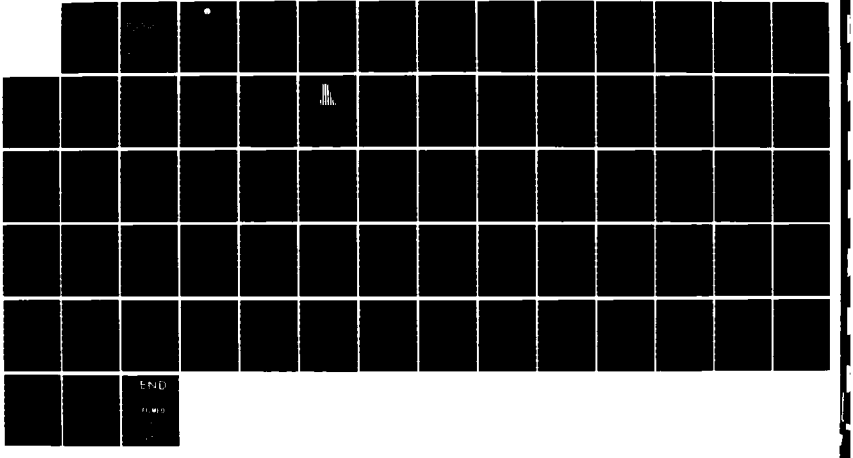

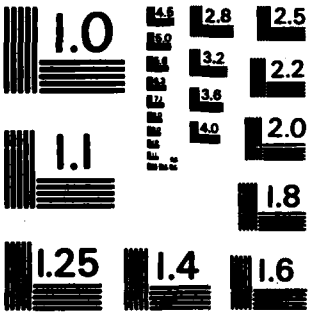

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SUBLETHAL GROWTH EFFECTS AND MORTALITY TO MARINE BIVALVES AND FISH FROM LONG-TERM EXPOSURE TO TRIBUTYLTIN

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ADMINISTRATIVE INFORMATION

The work described herein was performed by the Marine Environment Branch, Code 522, Naval Ocean Systems Center, for the David Taylor Naval Ship Research and Development Center.

Released by
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This study better defines the long-term toxicity and bioaccumulation potential of tributyltin released from antifouling paint to marine species. Because of the increasing evidence of the presence of tributyltin in environmental samples, the eastern oyster (Crassostrea *virginica)*, the bay mussel *(Mytilus edulis)*, and the flatfish *(Citharichthys stigmaeus)* were tested at low and sub-ppb tributyltin concentrations for a 65-67 day chronic test period. Additionally, the hatching success of fish eggs (California grunion, *Leuresthes tenuis)* exposed to tributyltin and subsequent larval fish survival at low tributyltin concentrations were also tested. Acute toxicity tests (96 hours) were conducted with mysid shrimp *(Acanthomysis sculpta)* to assess the toxicity of tributyltin to this sensitive marine species. The data clearly indicate sublethal growth effects and mortality to some marine species occur at tributyltin concentrations of 1 ppb and less.

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SUMMARY

The bay mussel (*Mytilus edulis*), the eastern oyster (*Crassostrea virginica*), and the marine flatfish (*Citharichthys stigmaeus*) were exposed to 0.04-1.89 ppb tributyltin to determine whether sublethal effects on growth parameters were evident after long-term exposure to tributyltin at trace levels. Mussels exposed to 0.31-0.73 ppb tributyltin for 66 days exhibited a significant decrease in shell length compared to specimens exposed to 0.12 ppb or less. Condition indices expressed as the whole body wet weight divided by the internal shell volume indicated a significant decrease in body weight in oysters exposed to 0.73-1.89 ppb tributyltin. Oysters exposed to 0.04-0.31 ppb exhibited a general decrease in condition index with increasing tributyltin concentration, although not statistically different from control values. Sublethal growth effects were not evident in marine flatfish exposed to 0.04-1.89 ppb tributyltin for 65 days.

Mortality was evident in bay mussel specimens after long-term exposure to tributyltin. A 66-day LC_{50} value of 0.97-ppb tributyltin was determined by probit analysis. Near complete survival was observed with eastern oysters exposed to 0.04-1.89 ppb tributyltin for 67 days. Poor control survival did not permit an estimation of low-level tributyltin toxicity to marine flatfish. Acute toxicity tests with the mysid shrimp (*Acanthomysis sculpta*) indicated juveniles were more sensitive to tributyltin than adults. Ninety-six hour LC_{50} values were 0.61 and 1.68 ppb, respectively. The data clearly indicate sublethal growth effects and mortality to some marine species occur at tributyltin concentrations of 1 ppb and less.

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INTRODUCTION

The presence of tin and organotins in environmental samples has been documented in several reports. Recently, organotins have been measured in marine plant and animal tissues (Ishu, 1982; Seidel et al., 1980; Tugrul et al., 1983). Tributyltin, specifically, has been measured in oyster tissues collected from areas where tributyltin was also measured in water samples (Waldock & Miller, 1983). With increasing evidence of the presence of tribultyltin in environmental samples, the need for accurate acute and chronic toxicity testing is emphasized.

Due in part to restraints in analytical capabilities, numerous reports exist in the literature where tributyltin toxicity data were reported as nominal estimated values, or as nonspecific measurements of total tin in the test solution. In either case, the utility of the toxicity data is questionable. Nominal values do not take into account experimental conditions such as adsorption of the toxicant to test container walls, resulting in an overestimate of the actual available toxicant in solution. Reports where nonspecific measurements of the toxicant were made do not address the chemical species in question (i.e., tributyltin is more toxic to marine species than monobutyltin, dibutyltin, or inorganic tin). Static renewal bioassay testing procedures may provide accurate estimates of a toxicant concentration if the test solution renewal period is brief, thus avoiding lengthy residence times and potential toxicant adsorption and degradation effects. Flowthrough bioassay testing accompanied by chemical speciation of the toxicant in solution is, however, a more desirable testing approach with respect to optimal testing conditions.

Several studies have recently appeared addressing the toxicity of tributyltin to freshwater and marine larval and adult organisms. Toxicity was frequently noted at low and less than 1-ppb levels. Primary productivity was reduced by 30 percent of that measured in controls after short-term exposure to tributyltin at less than 1 ppb with natural phytoplankton from lake waters (Wong et al., 1982). Tributyltin toxicity to marine zooplankton has been documented at 1 ppb or less (U'Ren, 1983). Acute tributyltin toxicity to larval marine species has been noted at concentrations ranging from several ppb to 0.1 ppb (Beaumont & Budd, 1984; Laughlin et al., 1984; Laughlin & French, 1980; Thain, 1983). Toxicity to adult marine species has generally been noted at higher concentrations, although total mortality has been reported after exposure to 4.8-ppb tributyltin for 5 days in some amphipod species (Laughlin et al., 1982). Avoidance of tributyltin at concentrations acutely toxic to several marine species has recently been demonstrated (Hall et al., 1984).

Few reports addressing chronic toxicity of tributyltin exist in the literature. Based on acute exposure data to freshwater fish, a "safe level" would lie in the range of approximately 0.12-0.27-ppb tributyltin (Chliamovitch & Kuhn, 1977). Exposure to tributyltin chloride (TBTCl) for a 110-day period at 1 ppb resulted in a 44-percent decrease in body weight in rainbow trout yolk sac fry. Biochemical changes in the hemoglobin content of blood and hyperplasia of liver cells were observed at 0.2 - 1.0-ppb TBTCl (Seinen et al., 1981). Oyster spat exposed to 0.15-ppb bis (tri-n-butyltin) oxide (TBTO) for 8 weeks exhibited a 70-percent reduction in weight relative to controls. No growth was observed in oysters exposed to 1.6-ppb TBTO for 8 weeks (Thain & Waldock, 1983).

Bioaccumulation has been reported recently in oysters exposed to TBTO at 0.15 and 1.25 ppb. Bioaccumulation factors ranged from 1,000-fold to 6,000-fold (Waldock et al., 1983). Two routes of bioaccumulation of TBTO have been reported in an estuarine crustacean (Evans & Laughlin, 1984). In short-term exposures, bioaccumulation via ingested food was quantitatively more important than direct uptake from water. TBTO has been reported bioaccumulated in whole fish by a factor of 2,600-fold. Factors as high as 52,000-fold were measured in liver tissues (Ward et al., 1981).

Recently, the accumulation and metabolism of tributyltin was reported in freshwater algae (Maguire et al., 1984). An accumulation factor of 3,000-fold was estimated for tributyltin. Approximately 50 percent of the accumulated tributyltin was metabolized to dibutyltin and small quantities of monobutyltin and inorganic tin.

To better define the long-term toxicity and bioaccumulation potential of tributyltin to marine species, the eastern oyster (*Crassostrea virginica*), the bay mussel (*Mytilus edulis*), and the flatfish (*Citharichthys stigmaeus*) were tested at low and sub-ppb tributyltin concentrations for a 65-67 day chronic test period. A 60-day bioaccumulation period was followed by a 30-day depuration phase. Length and weight measurements were taken during the course of the 65-67 day chronic test to determine whether sublethal indications of tributyltin toxicity had occurred. Juvenile and adult mysids (*Acanthomysis sculpta*) were tested for 96-hour periods to determine the acute toxicity of tributyltin to sensitive marine species.

Additionally, the hatching success of fish eggs (California grunion, *Leuresthes tenuis*) exposed to tributyltin and subsequent larval fish survival at low tributyltin concentrations were also tested (Newton et al., 1985).

METHODS AND MATERIALS

DESCRIPTION OF TESTING FACILITIES

The mobile bioassay laboratory used for FY 84 tributyltin toxicity testing consists of a 10- by 40-foot trailer. The interior is divided into two sections: the larger wet-lab area and the smaller dry-lab/office area. The trailer is wired for 110 and 220 Vac and is equipped with a 3-ton air-conditioning unit.

Six three-tiered racks, 7 by 2-1/2 feet, are positioned inside the wet lab for holding test containers. The upper and lower levels are equipped with removable, insulated, fiberglass water baths 75 by 32 by 6 inches deep. These are all plumbed to 1-1/2-inch polyvinyl chloride (PVC) plastic drain lines. The middle tier consists of a flat shelf with a 3/4-inch lip around the edge and a drain plumbed to the lower bath to control spills.

All racks are supplied with seawater by a 2-inch PVC supply line reduced to 1/2-inch feed lines. A low-pressure air supply is carried to each rack by 1/2-inch PVC pipe and a series of 5-way gang valves and vinyl air tubing. A high-volume/low-pressure supply of air is generated by an Aquanetics Model 202-P blower-type air pump equipped with an air intake particle filter.

Air temperature within the wet lab is controlled by the air-conditioning unit. Fresh air is continually drawn from outside the laboratory through a blower fan, which creates a slight positive pressure inside the lab. Lighting inside the wet lab is fluorescent and controlled by a timer on a 12-hour on/off cycle. A telephone warning system was installed in the dry-lab area to monitor possible electrical failures or interruptions in the seawater flowthrough system.

SEAWATER SYSTEM

The seawater supply to the mobile bioassay laboratory consists of two independent systems and was designed to deliver a dependable supply of seawater at constant pressure to the laboratory. Source water is drawn from offshore Point Loma. It passes through a series of screens and gravel filters to a 3,000-gallon open holding tank (Figure 1). This holding tank, under normal laboratory operating conditions, serves as a supply reservoir holding up to a 4-hour reserve of seawater. The reservoir supply is available in the event the main pumps must be shut down for repair or maintenance. It also acts as a settling and degaussing tank where large particles can settle out, particularly when the gravel filters in the main supply system are bypassed and unfiltered seawater saturated with gasses from the pumping action is collected.

Water for the laboratory is drawn from the holding tank through a 1-1/2-inch PVC pipe to a magnetic driven, plastic impeller pump, which transfers the water through a 1-inch PVC pipe to a 30-gallon covered header tank situated above the laboratory. The water level in this tank is maintained constant by an overflow pipe, which returns excess water to the holding tank. Seawater is fed directly into the lab from the header tank through a 2-inch PVC pipe by gravity flow, creating a constant pressure delivery.

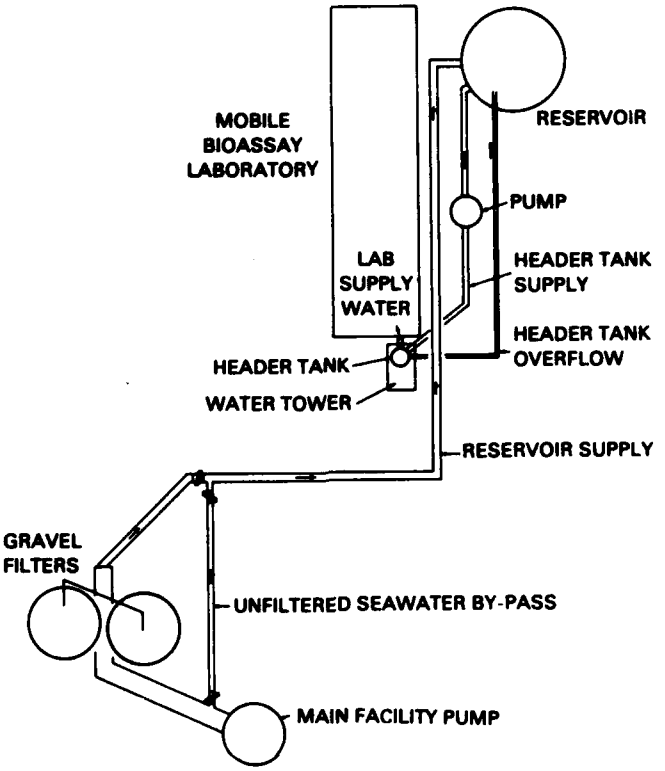

Figure 1. Seawater supply system and mobile bioassay laboratory.

TRIBUTYLTIN TOXICANT DELIVERY SYSTEM

Tributyltin was leached from painted plastic panels into flowing seawater delivered from the 30-gallon header tank. The tributyltin concentration was determined by the painted panel surface area exposed to seawater delivered at a fixed flow rate.

Painted panels were fixed vertically in place in plastic leaching troughs 6 feet long by 1 foot wide by 14 inches high. Water flows were directed so water would flow up the face of a panel and down the other side in troughs where one or more panels were used. Actual painted panel surface areas used in this study were 0.19, 0.48, 1.20, 3.0, and 7.5 square feet corresponding to test concentrations 1, 2, 3, 4, and 5. Seawater introduced directly from the 30-gallon source tank was used for the control solution. All panels were painted with SPC-954 antifouling paint provided by International Paints, Inc. (Figure 2).

Seawater flow rates across the panel surfaces were controlled by in-line flow meters positioned directly before the leaching troughs and set at 7-8 liters per minute. Seawater exposed to the painted panel surfaces was subsequently channeled over a series of wedged plastic surfaces to promote mixing and delivered to a 6-foot-long by 10-1/2-inch-wide by 6-1/2-inch-high mixing

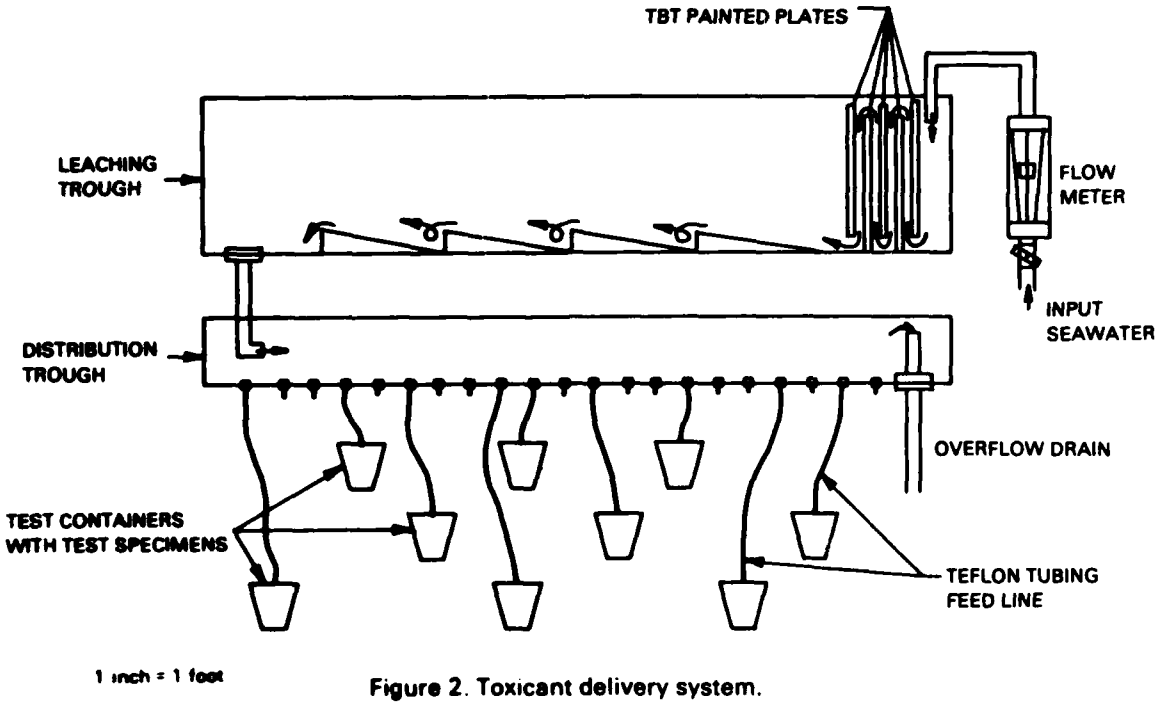

Figure 2. Toxicant delivery system.

trough fitted with exit ports connected to Teflon tubing. The Teflon tubes were individually directed to a specific 20-liter polycarbonate plastic test container fitted with an overflow line connected to the PVC drain system.

Flow rates through the Teflon tubing to the test containers were controlled by the insertion of small lengths of capillary glass tubing into the ends of the Teflon tubes. Tanks positioned on the top level of a given rack were fitted with a 1-inch-long 2-mm bore section. Tanks positioned on the middle level were fitted with a 2-inch-long 1.5-mm bore section. Tanks positioned on the bottom level were fitted with a 1-inch-long 1-mm bore section.

The flow rate to tanks on the top level was measured at 180 ml/minute. Tanks positioned on the middle and bottom levels had flow rates of 210 and 260 ml/minute, respectively. A diagram of the leaching trough and mixing trough positioned on each of five testing racks previously described is shown in Figure 2. The general arrangement of the bioassay trailer with the five test concentrations and the control treatment is shown in Figure 3.

SPECIMEN CARE

1. Acclimation Period

Fish, oysters, and mussels were held in a flowing seawater holding facility prior to transfer to the bioassay testing laboratory. Fish and oysters were bought from commercial sources, while mussels were collected in San Diego Bay from an area remote from known tributyltin input sources.

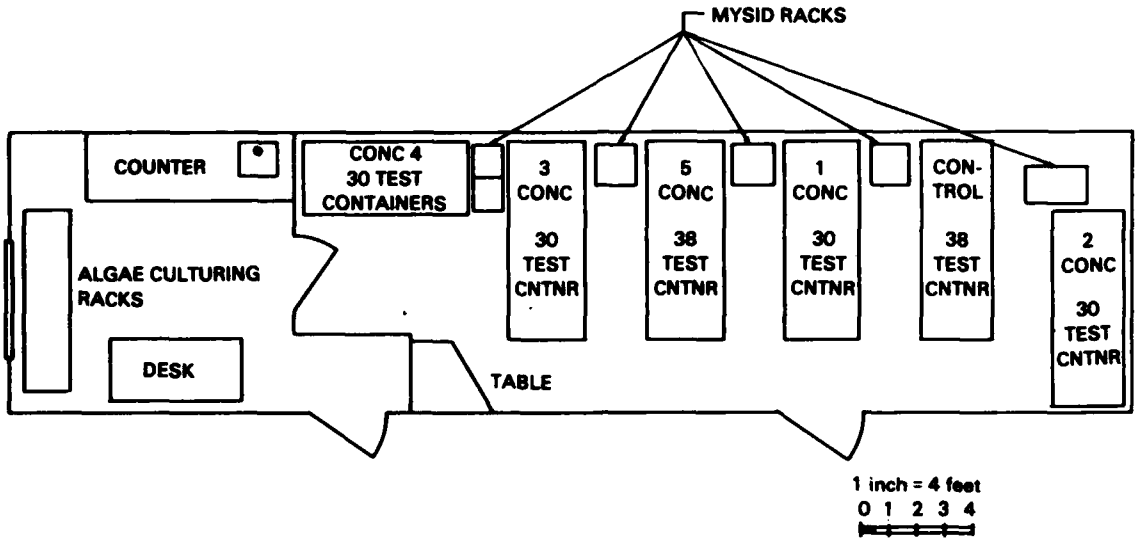

Figure 3. Mobile bioassay laboratory testing facility arrangement.

Mussels and oysters were transferred to the testing facility and further acclimated for a 7-day period. During this secondary acclimation phase, initial measurements and weights were made on individual specimens. Fish were transferred to the testing facility and acclimated for a 10-day period. Initial measurements and weights were recorded during the first and second test days. Due to the large number of test species, weights and measurements could not be completed in 1 day. Initial length, width, and weight data were, therefore, reported as if recorded on the first test day. Subsequent weights and length and width measurements were also reported for a given test day if the process could not be completed in a single day.

During the acclimation period, dead specimens were removed and replaced where necessary to maintain equal numbers of test species. Although fish testing containers were covered during the course of the chronic testing phase, some specimens jumped out where lids were left partially open. Specimens were counted and replaced where necessary during the acclimation phase. During the testing phase, specimens that had jumped out of test tanks were not replaced. Only six such instances (2 percent) were encountered and, therefore, did not adversely affect the test results.

2. Feeding

Mussels and oysters were fed cultured algae (*Duniella sp.*, *Rhodomonas sp.*, and *Tahitian Isocrysis sp.*) intermittently during the acclimation and testing phases, in addition to the phytoplankton present in the filtered seawater supplied by the flowthrough system. Feeding with cultured algae was terminated after the 10th test day due to difficulties in maintaining mass

algal cultures. At this time the seawater filters were bypassed to supply unfiltered seawater to the testing facility on a 12-hour on/off cycle as a food source for the bivalve species for the duration of the test.

Continuous flowthrough of unfiltered seawater was not possible due to fouling of the glass capillary tubes regulating the flow of water through the Teflon source lines to the testing tanks. Measurement of the chlorophyll content of the seawater passing through the filter system compared to raw unfiltered seawater indicated approximately 70 percent of the phytoplankton present in unfiltered seawater was removed by the filtering process. The 12-hour on/off, unfiltered/filtered seawater cycle, therefore, represented an approximate 38-percent reduction in the amount of natural phytoplankton available to the bivalves under flowthrough conditions.

Fish were fed a diet of dried flake food and frozen euphausiids. In the morning a Tetra-Min flake food, SD-80, was fed, in slight excess, to all fish. A ration averaging 1.1 gm/tank was fed to the chronic test fish, and a 2.2 gm/tank ration was fed to the accumulation test fish. During the afternoon feeding, cakes of frozen euphausiids were thawed into a soup, and rations averaging 1.7 and 3.2 gm damp-weight/tank were fed to chronic and accumulation test fish, respectively. Feeding with flake food was discontinued in the chronic test after the 15th test day due to persistence of excess food, which may have been responsible for poor testing conditions regarding bacterial growth and, possibly, high ammonia levels. Subsequently, chronic test fish were fed euphausiids at both morning and afternoon feeding intervals.

All feeding rations were slightly in excess, and the tanks were cleaned of remaining food regularly. As specimens were removed through sampling and mortality, the frequency of tank cleaning was increased since feeding rations were kept constant throughout the testing phase.

PRETEST-SPECIMEN DISTRIBUTION

1. Chronic Test

A seawater control and five test concentrations were used to determine long-term and sublethal tributyltin toxicity to flatfish and bivalves. Five replicate test tanks with 10 fish, 10 oysters, or 10 mussels per tank were placed in racks corresponding to a specific test concentration or control. A specific level of the three-tiered rack was dedicated to a specific test species. Mussels and oysters were individually marked with waterproof ink or marking cement so individual records of length, width, and weight could be kept. Individuals were marked in sequence from 1 to 10.

Specimens were introduced into the test tanks across concentrations by replicate or pairs of replicates. All test concentrations and the control were, therefore, loaded with test specimens from the test population at the same rate. Unfortunately, due to the large number of specimens tested, individuals of identical size could not be obtained. This was especially true of fish and mussel species collected from natural populations.

Potential dissimilarities in specimen size (length) among replicate tanks within concentrations and among concentrations at the beginning of the

test for fish, mussel, and oyster data were tested by a nested one-way analysis of variance (ANOVA) approach provided by the Omnibase data processing package (Omnibase data management system, 1984). Generally, significant differences in specimen size were present among replicate tanks within concentrations ($p < 0.05$). Subsequent statistical testing procedures, therefore, considered test tanks as replicates. Individual specimens could have been considered replicates, if no differences in beginning specimen size had been found among replicates within a given concentration. This would have increased the degrees of freedom and the power of the statistical testing.

Differences in specimen size among all concentrations tested (0, 1, 2, 3, 4, and 5) were not significant at the beginning of the test ($p > 0.05$) for fish and mussel data. Therefore, no single test concentration favored a particular specimen size that could have influenced test results in both mortality and growth parameters. Oyster specimen lengths were, however, significantly different (ANOVA, $p < 0.05$) at the beginning of the test. Multiple range testing indicated that oysters in the control group were significantly different ($p = 0.05$), having a greater mean shell length than specimens in the treatment concentrations. Subsequent statistical testing, therefore, was not applied to potential changes in oyster growth. The size distributions of the test specimen populations used in the chronic test are shown in Figures 4, 5, and 6.

2. Bioaccumulation Test

Fish, oyster, and mussel specimens were introduced into test tanks in the same manner as the specimens used in the chronic test. Fifteen fish and mussels were tested per replicate tank, while 27 oysters per tank were tested. Only three test concentrations and the seawater control were used in this test. The test concentrations were 1, 3, and 5.

Individual specimens were not marked or measured during the course of the test. Mortality data were recorded but not used for estimates of toxicity since the purpose of this test was to assess the bioaccumulation of tributyltin in marine fish and bivalves. The sampling schedule and specific testing methods employed in this study are described in detail in a Naval Ocean Systems Center (NOSC) technical document presently in preparation.

MEASUREMENT METHODS

Specimens were weighed and measured at four intervals during the chronic bioassay test. Measurements were made with a hand-held micrometer to 0.01 cm. Specimens were weighed on a Fisher Model 7210 top-loading balance to 0.01 gm.

The procedure for weighing fish consisted of capturing and transferring individuals to a pre-tarred glass dish partially filled with seawater. Fish were lightly blotted on soft paper towels prior to weighing; therefore, the weights recorded were wet weights. Immediately after weighing, fish were measured by placing a ruler under the dish and orienting the fish so a measurement of the length, from the tip of the head to the hypural plate just prior to the origin of the fin rays, was possible.

Mussels and oysters were weighed and measured by individually removing the specimens and blotting them with a paper towel. Individuals were then immediately placed in pre-tarred plastic beakers partially filled with seawater. Any

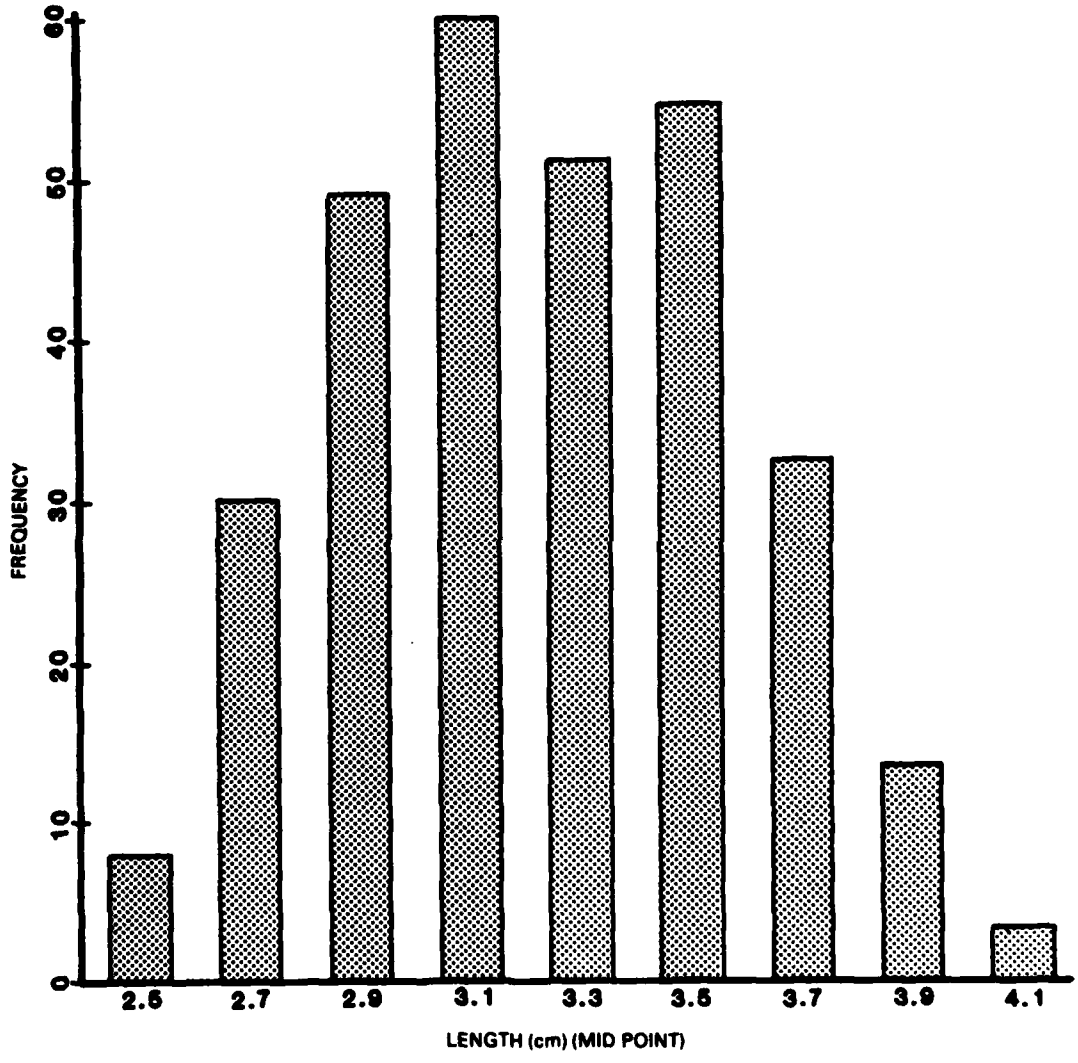

Figure 4. Length distribution of mussels used in chronic toxicity test at time = 0 for all test concentrations (frequency = no. of individuals).

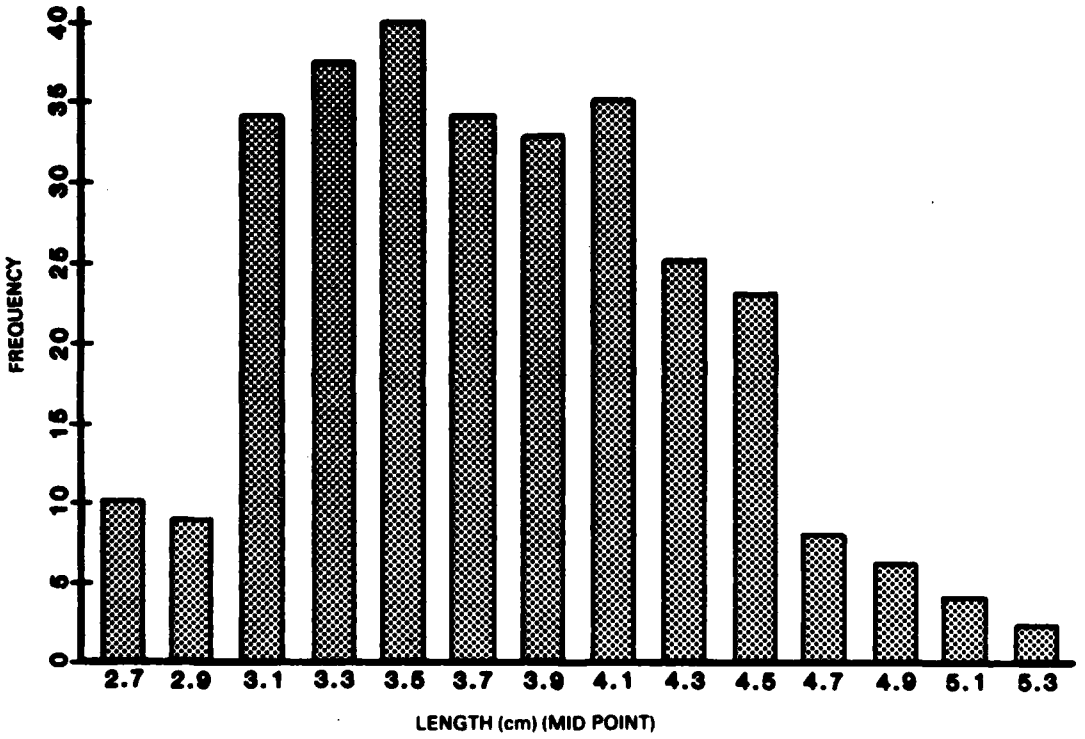

Figure 5. Length distribution of oysters used in chronic toxicity test at time = 0 for all test concentrations (frequency = no. of individuals).

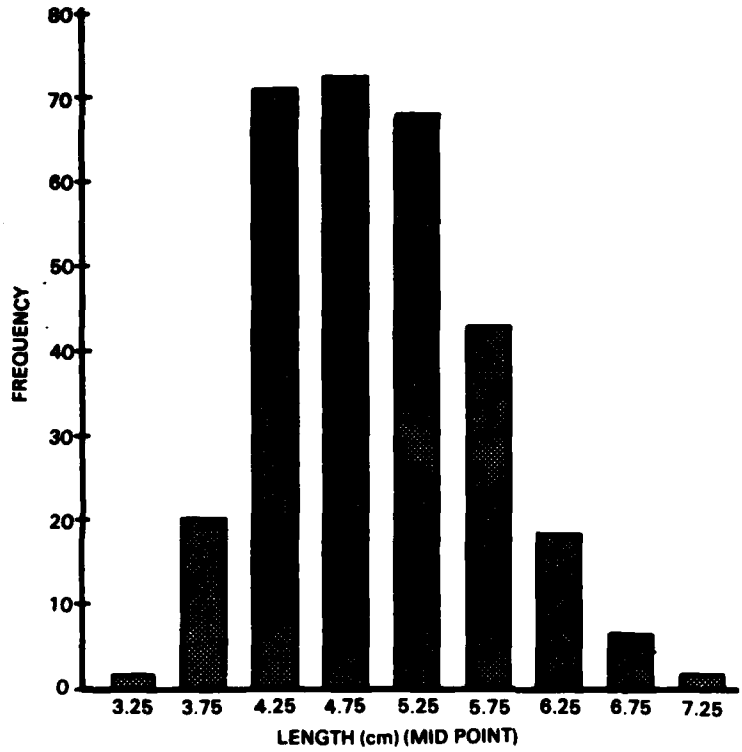

Figure 6. Length distribution of fish used in chronic toxicity test at time = 0 for all test concentrations (frequency = no. of individuals).

specimens that had captured air in their valves could be detected by this procedure. Lengths and widths of shells were then measured with a hand-held micrometer. Byssus threads were not removed between measurement intervals, although at the beginning of the experiment excess byssus threads and detritus were cleaned from individuals prior to marking.

An estimate of the relative weighing and measurement precision expressed as the coefficient of variation (one standard deviation divided by the mean) was calculated for all three species tested. Several replicate weight, length, and width measurements were taken on a single specimen to generate means and standard deviations. A coefficient of variation of 0.8 percent was determined for oyster weight measurements. The coefficients of variation for oyster length and width measurements were 0.4 and 0.87 percent, respectively. Mussel weight measurements exhibited a coefficient of variation of 1.4 percent. The coefficients of variation for length and width measurements were 0.16 and 0.2 percent, respectively. Fish weight and length measurements had coefficients of variation of 1.9 and 1.1 percent, respectively. Coefficients of variation determined for weighing and measuring methods used in this study were small (frequently less than 1 percent) indicating these procedures were capable of determining slight changes in body weight, length, or width.

CONDITION INDEX

In addition to length, width, and weight measurements, a condition index was calculated for mussels and oysters after the long-term toxicity test was concluded. The index used has previously been described (Baird, 1957) and is defined as the ratio of the wet weight of body meat to the shell cavity volume.

Mussel and oyster body volumes were measured by placing the specimens in a 250-ml chamber equipped with a side tube approximately 120 cm long extending from the chamber base at an angle of 7.5°. Volume displacements in the 250-ml chamber were, hence, magnified and could be read to 0.18 ml.

Several repetitive measurements were made on the same oyster specimen to determine the relative volume measurement precision expressed as the coefficient of variation. Oysters were selected that had shell volumes near 1 and 4 ml, representative of the low and high volume measurements determined for all bivalves measured. Ten repetitive volume measurements of an oyster shell with a shell volume near 1 ml resulted in a mean of 1.34 ml with a standard deviation of 0.177 ml. The coefficient of variation determined from the standard deviation divided by the mean was 13.2 percent. An oyster shell having a volume near 4 ml exhibited a mean volume of 3.81 ml with a standard deviation of 0.137 ml after 10 successive measurements. The corresponding coefficient of variation determined for this larger specimen was 3.6 percent, indicating measurement precision may have been slightly better with larger specimens. An estimation of volume measurement precision was not performed with mussels, but was assumed to be similar to that determined for oysters.

The actual measurement procedure involved making an initial measurement of the entire specimen including the shell and shell cavity volume, removing the body meat and recording the wet meat weight after excess water and body fluid was blotted, and then taking a second volume measurement of the empty shell. The difference between the first and second volume measurement determines the shell cavity volume used in the condition index calculation.

Volume measurements read from the water meniscus position in the side tube in millimeters from a scale fixed to the tube were regressed against a known volume displacement in the chamber so all measurements could be expressed in milliliters. A given volume displacement was determined by reading the meniscus in the side tube before and after the bivalve shell was placed in the chamber.

The calculation for the condition index was performed by using the following formula:

$$\text{condition index} = \frac{\text{body meat wet weight (gms)}}{\frac{[(\text{scvol2}-\text{scvol1})-(b)]}{m} - \frac{[(\text{svol2}-\text{svol1})-(b)]}{m}}$$

where:

scvol1 = initial volume reading of the entire organism
scvol2 = final volume reading of the entire organism
svol1 = initial volume reading of the shell only
svol2 = final volume reading of the shell only
m = slope of the calibration regression equation
b = y-intercept of the calibration regression equation

Condition indices calculated for oysters and mussels ranged from 0.04 to 0.59. Larger condition indices are found in specimens where more shell cavity is filled by tissue and are assumed to be characteristic of healthy specimens. Small condition index values indicate a lesser amount of tissue per shell cavity volume is present.

STATISTICAL TESTING

Statistical testing of changes in specimen weights, lengths, and widths for oysters and mussels among test concentrations during the course of the test was accomplished by the use of a one-way fixed effect ANOVA model with duplicates. Analysis of variance testing of fish growth data, however, did not employ duplicates. Since individual records of specimens were not kept over the length of the testing period, the means of growth measurements from a given test tank within a concentration were used.

The test designs were balanced, making it unnecessary to test for the assumptions of normality or homoscedasticity (a condition where individual cell variances are equal). Therefore, transformations of the data were not made. Each significant ANOVA ($p < 0.05$) was followed with a Student-Newman-Keuls Multiple Range Test (MRT). The MRT aids in determining which concentrations had different means, thereby contributing to the significant ANOVA test.

CHEMICAL MONITORING OF TRIBUTYLTIN AND WATER QUALITY MEASUREMENT

Tributyltin was measured in test concentrations by hydride derivatization followed by atomic absorption detection. The analysis technique was the same as that described elsewhere (Valkirs et al., 1985), with some minor modification. The column packing material was changed to 3-percent OV1 Chromosorb, permitting better peak resolution than the quartz wool previously used.

Tributyltin measurements made during the chronic test are shown in Figure 7. Mean tributyltin concentrations in ppb were calculated for the entire testing period (Table 1). The mean tributyltin value for concentration 1 was 0.04 ppb. Concentrations 2, 3, 4, and 5 had values of 0.13, 0.31, 0.73, and 1.89 ppb, respectively. Control samples were periodically taken along with test concentration samples and analyzed. No tributyltin was detected in control samples (detection limit = 0.01 ppb).

Samples from test solutions were generally collected once a week from the distribution trough above the test tanks. To verify the accuracy of analytical results, a tributyltin reference sample was analyzed with each set of test samples. A large volume of test solution from test concentration 2 was collected in a single container and poured into separate 500-ml polycarbonate plastic bottles and frozen. These samples were used as the reference sample for subsequent analytical sessions. Previously, natural seawater samples from San Diego Bay were found to be stable during frozen storage. The results of the frozen reference sample analysis are listed in Table 1.

Water quality data were taken during the chronic test to ensure testing conditions were appropriate. Water quality data were taken on the third day of the test in all test tanks and, periodically, during the rest of the testing period in two test concentrations. These data are presented in Appendix A. Ammonia measurements were made once, on test day 63, in several test tanks. Because the flowthrough exchange rate in the test tanks occurred rapidly, high ammonia levels were not anticipated. Ammonia in seawater was determined as nitrogen in micrograms/liter by methods described in Strickland and Parsons (1972). A detection limit of 0.1 micrograms/liter was determined. The results of ammonia determinations are presented in Appendix B.

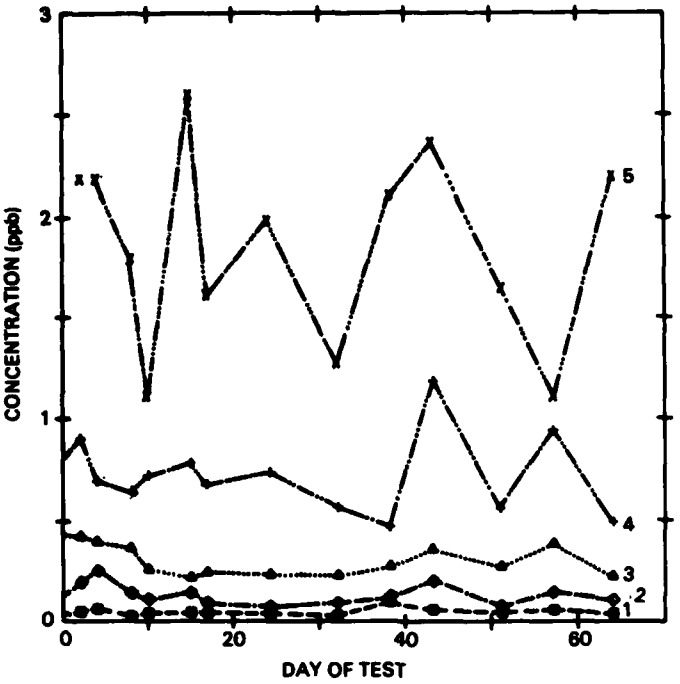

Figure 7. Tributyltin concentration measured in test solutions 1 — 5.

Seawater samples were taken from test concentrations and analyzed for copper by atomic absorption spectrometry. Copper is a minor component of the SPC-953 antifouling paint used in this study. The possible presence of copper in the test solutions is of interest because of potential synergistic toxic effects with tributyltin. Copper samples were collected from all test solutions on day 31 of the chronic test. The results of the copper analysis are presented in Appendix C.

MYSID TEST FACILITIES AND CONDITIONS

Mysids were tested with the same tributyltin toxicant source used in the chronic bioassay study. However, some modifications of the testing facilities were made to accommodate the small size of this species. The flowthrough test containers used for all mysid tests consisted of polycarbonate plastic bottles equipped with overflow drain ports covered with 202-micron Nitex screen. The effective holding volume of the containers is approximately 550 mls. These containers are situated in a 20-liter tray fitted with a drain to remove overflow water. The test containers are exposed to toxicant via a polycarbonate plastic reservoir positioned above. Seawater with tributyltin toxicant flows through constricted Pasture pipets that regulate the flow rate. Seawater is directed to the test chambers by Teflon tubes positioned at the ends of the Pasture pipets and descends to the bottom of the test chambers. The reservoir tank, connected directly to the leaching troughs, supplies the chronic test tanks through Teflon tubing.

Flow rates to the mysid test containers averaged 30 ml/min, which is equivalent to approximately 78 total seawater exchange volumes per 24-hour period.

Table 1. Reference sample analysis of frozen water samples and mean test concentrations from treatments 1-5.

Test day	Calendar day	Tributyltin µg/liter
8	4/24/84	0.13
10	4/26/84	0.13
15	5/01/84	0.14
17	5/03/84	0.11
24	5/10/84	0.13
32	5/18/84	0.09
38	5/24/84	0.13
43	5/29/84	0.10
51	6/06/84	0.11
57	6/12/84	0.10
58	6/13/84	0.10
64	6/19/84	0.10

Mean tributyltin concentration = 0.11 µg/liter
Standard deviation = 0.017
Coefficient of variation = 15.5 percent

Mean tributyltin test concentration µg/liter

Treatment	$\bar{X}$	STDV	CV
1	0.04	0.018	45.0 percent
2	0.13	0.057	43.8 percent
3	0.31	0.079	25.5 percent
4	0.73	0.195	26.7 percent
5	1.89	0.479	25.3 percent

1. Juvenile Mysid Test

Juvenile mysids were obtained from adult, gravid female *Acanthomysis sculpta* collected from the kelp beds off Point Loma. Freshly collected adults were acclimated to laboratory conditions, then sorted. Groups of five females were placed in 150-ml aerated glass crystallizing dishes. Water was replaced in each dish once a day or when the water became cloudy.

Each morning and afternoon, dishes were examined for newly hatched juveniles, which were separated from the adults and placed in a 700-ml static holding container with aeration. When enough juveniles were collected to fill an entire replicate across all concentrations, the juveniles were distributed into the appropriate flowthrough test containers. Ten individuals were placed in each of five replicate containers. In this way, the start of the test was staggered throughout 6 days, and the age of the juveniles at time 0 ranged from 1 to 3 days. The test was started in this manner to begin testing juvenile mysids as soon as possible after hatching.

Daily observations in each test container were made, and the number of dead individuals was recorded. Because of their small size and active movement about the test container, accurate counts of live individuals during the first part of the test were not possible without disturbing the specimens. As the juveniles grew and became more visible, efforts were made to count live as well as dead specimens.

Feeding was accomplished by introducing live, freshly hatched brine shrimp into each test container every other day for the first 10 test days. All food introduced was apparently eaten, and the feeding ration was, therefore, increased to once daily.

2. Adult Mysid Test

Mysids used in the adult *Acanthomysis sculpta* acute toxicity test were collected in the same area, but on separate days than when juvenile mysid breeding adults were collected. Adults collected for the adult acute toxicity test were sorted, and roughly 50 individuals were placed in ten 20-liter holding tanks supplied with flowthrough seawater. After a 24-hour holding period, groups of 10 mysids were counted and placed in test containers. Five replicates of 10 individuals per test concentration were used.

Observations were made daily on live and dead individuals. Dead mysids were removed and measured. The number of gravid females present in each test container at the beginning of the test was recorded. The presence of juveniles was recorded throughout the test.

Adult mysids were fed *Artemia salina* nauplii daily at a ration of 10-cc nauplii per test container. Excess detritus was removed from containers when necessary.

RESULTS AND DISCUSSION

SUBLETHAL GROWTH

1. Bivalves

A. Mussels

Growth data for mussels expressed as the difference in mean shell length, width, and whole body weight (soft tissues plus shell) between measurement intervals are shown in Figures 8, 9, and 10, respectively. Since only six mussels survived the 66-day testing period in concentration 5, subsequent statistical testing of growth parameters was conducted with concentrations 1, 2, 3, 4, and the control. No significant differences in shell width or whole body weight were detected among concentrations after the 66-day testing period (ANOVA $p > 0.05$, 2-tailed test). However, a significant difference in the length of mussel shells was detected.

Multiple range testing of mean shell lengths with the Student-Newman-Keuls test (Zar, 1974) indicated mean shell lengths were similar among concentrations 1, 2, and the control. However, the control treatment and concentrations 3 and 4 were significantly different ($p < 0.05$). A decrease in shell length was observed after the 66-day testing period in concentrations 3 and 4 (Figure 8). The observed differences in shell length were, therefore, well-ordered relative to test concentrations since mean shell lengths of mussels tested in concentrations 3 and 4 were statistically different from those in the control and followed a general trend of decreasing shell length with exposure to higher test concentrations.

Statistically significant growth in shell length was measurable by our technique (coefficient of variation = 0.16 percent; therefore, a mean shell length of 3.3 cm is measured ± 0.005 cm). Little growth was observed during the 66-day testing period (Figure 8). A maximum increase in mean shell length of 0.05 cm was recorded in the control. The restricted availability of natural phytoplankton, necessitated by the design of the flowthrough seawater system to avoid fouling of the small capillary delivery tubes, was a probable contributing factor. Differences (ANOVA, $p < 0.05$) detected in shell lengths among concentrations were based on changes of 1 percent or slightly more and supported an overall decrease in shell growth from the control treatment to test concentration 3 following the order of control, 1, 2, and 3 among test concentrations where specimen survival was high (90 percent or better). The largest decrease in shell length was noted in test concentration 4, where survival was near 50 percent after the 66-day exposure period.

We are not aware of any studies where the effects of tributyltin exposure on adult mussel growth have been investigated. Recently, effects on the growth and survival of *Mytilus edulis* larvae have been reported (Beaumont & Budd, 1984). Growth in larvae exposed to 0.1 ppb-tributyltin was significantly reduced ($p < 0.01$) from that seen in controls over a 15-day exposure period (Beaumont & Budd, 1984).

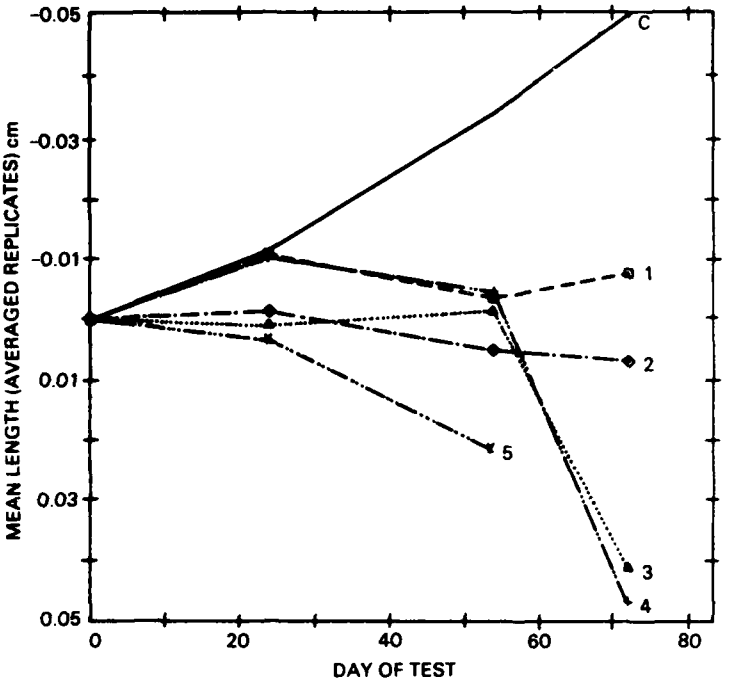

Figure 8. Mussel shell length change recorded during chronic toxicity testing.

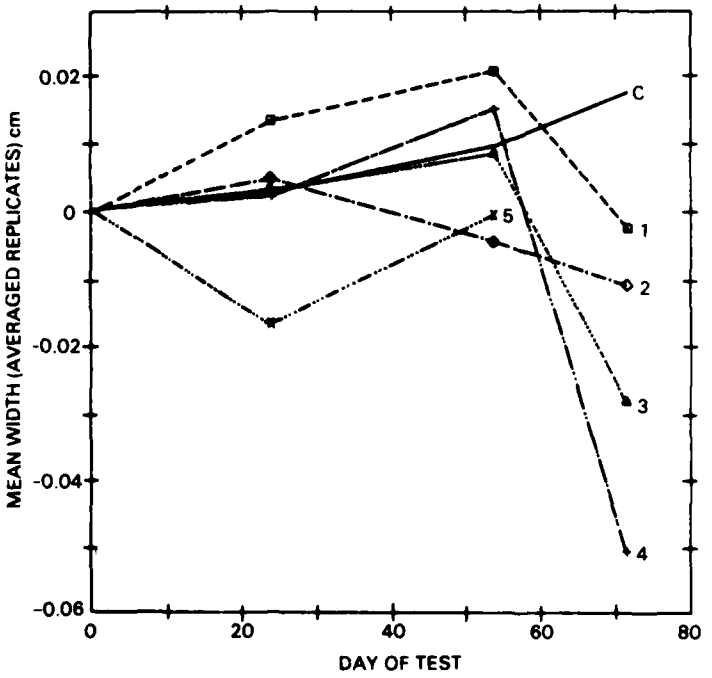

Figure 9. Mussel shell width change recorded during chronic toxicity testing.

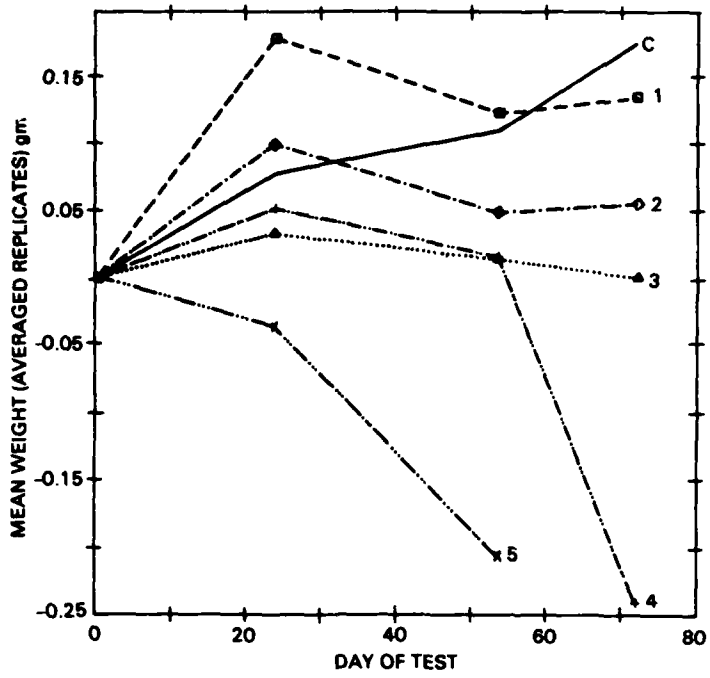

Figure 10. Mussel whole body weight change recorded during chronic toxicity testing.

A condition index expressed as the ratio of the wet body meat weight divided by the internal shell volume was calculated for mussels surviving the long-term testing period. No significant difference (ANOVA, $p = 0.05$) among test concentrations was observed. Condition indices for mussels tested in the control treatment and test concentrations 1-5 are shown in Figure 11. Mussels exposed to near-sublethal tributyltin concentrations (1 and 2), and those in the control treatment exhibited similar condition indices to those representative of mussels tested at higher tributyltin concentrations (3, 4, and 5).

B. Oysters

Since the control treatment was significantly different from the test concentrations with respect to initial lengths of specimens and, hence, could potentially have influenced growth parameters, differences in length, width, and weight were not compared statistically. Apparent changes in length, width, and weight are, therefore, discussed in terms of graphical trends. The data are presented in Appendix D. Specimen weight appeared to have increased graphically in all concentrations tested. Growth in terms of length and width was again minor (less than 5 percent of the mean length and width of specimens measured at the beginning of the test) and likely influenced by the restricted food supply.

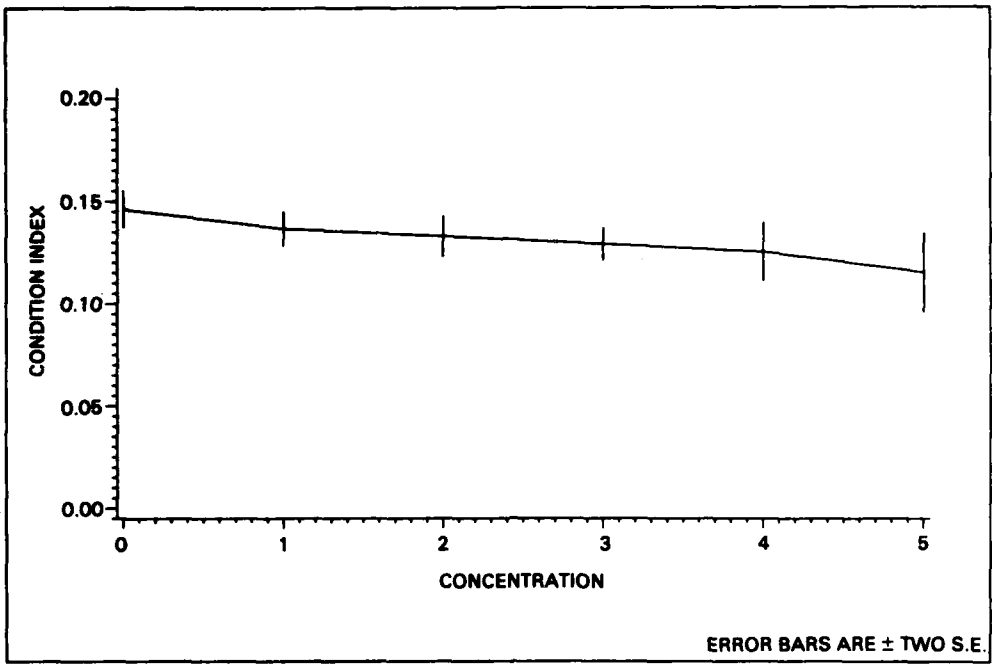

Figure 11. Mussel condition indices and test concentrations.

Little information is available on the effects of tributyltin on oyster growth rates. Excessive shell thickening and irregular growth was observed in field and laboratory studies with *Crassostrea gigas* (Alzieu, 1981; Alzieu et al., 1982). Oysters taken from reference areas and placed in harbors where vessels with organotin antifouling paints were present developed shell abnormalities, which ceased after the oysters were moved back to the original reference sites. Laboratory studies confirmed abnormal shell calcification in oysters exposed to an estimated 0.2-ppb tributyltin.

Recently, the effects of suspended sediment and tributyltin on the growth of *Crassostrea gigas* spat (newly spawned oysters) were investigated at low (0.15 ppb) tributyltin concentrations (Thain & Waldock, 1983). Oyster spat exposed to 0.15-ppb tributyltin for 8 weeks showed a reduction in weight of approximately 70 percent compared to control specimens. Increases of 100-125 percent from initial weights were seen in oyster spat exposed to 30-75 mg/liter sediment without tributyltin present. Exposure to 1.6-ppb tributyltin resulted in no weight increase at the end of the testing period. Growth in the length of shells expressed as the percent of the initial length ranged from 0.2 to -0.1 percent at tributyltin concentrations of 0.15 and 1.5 ppb, respectively. A maximum increase of 1.4 percent was recorded where oyster spat were exposed to 0.15-ppb tributyltin and 30-mg/liter sediment. Controls exhibited a 4.7-percent length increase; increases in weight were predominantly due to thickening of the shell valves.

Our results are similar with respect to increases in oyster shell length. The maximum increase in shell length was observed in controls, which grew by an average of approximately 0.05 cm (1.2 percent of the initial average size of the controls) during the 67-day testing period. Oysters exposed to 1.89-ppb tributyltin grew by an average of 0.4 percent of their initial average lengths. All concentrations exhibited positive growth in terms of increased length over the course of the test, although large variations were noted within the 67-day testing period. Increases in length did not follow a trend with increasing tributyltin concentrations.

Reduced food availability and differences in salinity (normally approximately 26-28 ppt where the oysters were cultured compared to 32 ppt locally) may have restricted growth in oysters used in the study. Growth (4.7-percent increase) in terms of shell length was considered normal in control oyster spat (*Crassostrea gigas*) fed cultured algae twice daily (Thain & Waldock, 1983). Possibly, a 67-day period was inadequate for observation of an increase in shell length greater than 2-3 percent in *Crassostrea virginica* under the testing conditions used in our study.

Condition indices were calculated for oysters surviving the long-term toxicity testing phase. These data are presented in Figure 12. Condition indices were also calculated for a frozen subsample of 11 specimens from the oyster population used for the long-term toxicity test. This oyster subsample was removed from the oyster group used in the long-term test prior to initiating the test and, hence, represents a group not exposed to experimental conditions for the 67-day period. Condition indices calculated for these pretest specimens might be considered more closely representative of the natural oyster population the test specimens were drawn from and not subject to experimental conditions such as limited food availability.

Differences in condition indices among the control, the pretest subsample (reported as concentration 6 in Figure 12), and test concentrations 1, 2, 3, 4, and 5 were tested statistically (one-way ANOVA) and found to be significant ($p < 0.05$). A general decrease in condition index with increasing tributyltin concentrations was evident (Figure 12). Multiple range testing demonstrated that mean condition indices calculated for concentrations 4 and 5 were significantly smaller than those calculated for the control, the population subsample, and concentrations 1, 2, and 3 ($p < 0.05$). Since fewer observations were included in the population subsample group, differences in condition indices among concentrations were also tested without the population subsample data. F-statistics were virtually identical (15.38 and 15.13, respectively) and condition indices calculated for concentrations 4 and 5 were again significantly different from the control and concentrations 1, 2, and 3 ($p < 0.05$).

Earlier testing of oyster lengths at the beginning of the long-term toxicity testing phase had indicated oysters in the control treatment had significantly larger shell lengths than oysters in the test concentrations. Additionally, some evidence has been presented for an association of oyster condition index with shell size (Baird, 1957). To determine if the shell lengths of oysters used in this study were associated with condition indices, a nested analysis of covariance (ANCOVA) was performed using final shell lengths as a covariant with concentration. No significant association with shell

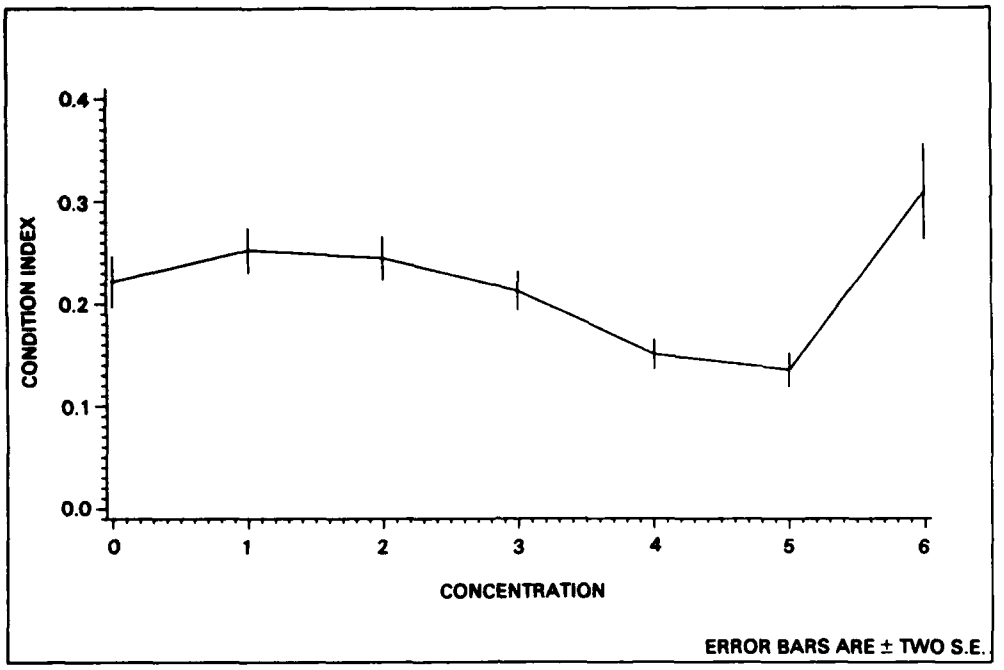

Figure 12. Oyster condition indices and test concentrations (concentration 6 represents a population subsample removed from the specimen population prior to testing).

lengths and test concentrations was found ($p > 0.05$). A scatter plot of condition index versus shell length for the control, population subsample, and test concentrations 1, 2, 3, 4, and 5 demonstrated a high degree of variability in the data supporting a nonsignificant association of condition index with shell length.

Condition indices calculated for oysters (*Crassostrea virginica*) exposed to 0.04-1.89-ppb tributyltin in our study are similar to those reported for *Crassostrea gigas* (Thain & Waldock, 1983), where oyster condition indices were determined by the same method. Values reported in their 8-week study ranged from 51-23 (less by a multiplication factor of 100 for direct comparison to values reported in this study) at tributyltin exposures of 0.15-1.60 ppb, respectively. Although statistical analysis was not performed with their data, the results are comparable to those presented in this report. In both studies, exposure to tributyltin at higher concentrations (1.60 and 1.89 ppb) clearly resulted in lower condition indices in oysters compared to indices determined for oysters exposed to low tributyltin concentrations (0.15 and 0.04-0.13 ppb).

The generally larger condition indice values reported for *Crassostrea gigas* (Thain & Waldock, 1983), compared to those determined for *Crassostrea virginica* in this report, may be due to species-dependent metabolic differences or tissue preparation methods. Weights expressed as wet tissue may be consistently determined within a given study. However, slight variations in technique, associated with the degree of tissue blotting for example, may make strict comparisons between studies difficult.

2. Fish

Growth data for flatfish (*Citharichthys stigmaeus*) are shown in Figures 13 and 14. Significant differences in growth, with respect to changes in length or weight during the course of the test, were not found among test concentrations (ANOVA, $p > 0.05$). Changes in length and weight were positive throughout the testing period and were not concentration related, indicating the tributyltin concentrations tested (0.04-1.89 ppb) did not affect sublethal growth relative to controls.

A loss in weight of 6.65 percent in freshwater fish (*Tilapia mossambica*) exposed to 8-ppb TBTO for a 5-week period has been reported (Matthiessen, 1974). Specimens exposed to 5 ppb for 5 weeks exhibited a weight increase of 7.46 percent, while control specimens increased in weight by 11.72 percent. A 44-percent decrease in body weight has been observed in rainbow trout (*Salmo gairdneri*) yolk sac fry exposed to 1-ppb tributyltin for 110 days compared to control specimens (Seinen et al., 1981). Fish exposed to 0.2 ppb exhibited less pronounced growth retardation. Body weights were significantly lower (approximately 20 percent) than those of controls only near the end of the 110-day testing period. Growth in terms of length and weight was not significantly different from controls with saltwater fish (*Cyprinodon variegatus*) exposed to TBTO for 167 days at 1 ppb (Ward et al., 1981). Apparently, species-specific differences may exist between fish, with respect to sensitivity to sublethal concentrations of tributyltin. Additionally, comparisons are difficult to make between sublethal toxicity studies by various investigators due to potential problems with experimental design and execution at low tributyltin levels. Our results indicate growth in marine flatfish (*Citharichthys stigmaeus*) is not adversely affected by concentrations of tributyltin ranging from 0.04-1.89 ppb.

MORTALITY

1. Fish

The cumulative percent mortality during the course of the chronic test is shown in Figure 15. More than 50 percent of the specimens tested in concentrations 1, 3, and 4 died during the 65-day testing period. Mortality was not, however, concentration related. Specimens in the control and highest concentration tested (concentration 5=1.89 ppb) exhibited similar cumulative mortalities of approximately 47-48 percent. Cumulative percent mortality was highest (approximately 75 percent) in concentration 3 (0.31 ppb). The high mortality observed in the controls may, in part, have been due to excess food and consequent bacterial activity in the test tanks, or possibly it was a consequence of disease. Such experimental conditions may have contributed to mortality observed in the other test concentrations as well, although flowthrough seawater exchange rates were large (14-20 test tank volumes per 24 hours) and, presumably, could have mitigated waste accumulation.

Numerous studies exist in the literature where tributyltin toxicity to fish species was tested by various bioassay testing procedures. The results of many of these earlier investigations are suspect due to limited testing procedures, notably in chemical measurement of the available toxicant in solution. Unfortunately, concentration-related toxicity was not observed in this study, possibly due to testing conditions; thus, it was not possible to calculate a long-term LC_{50} value.

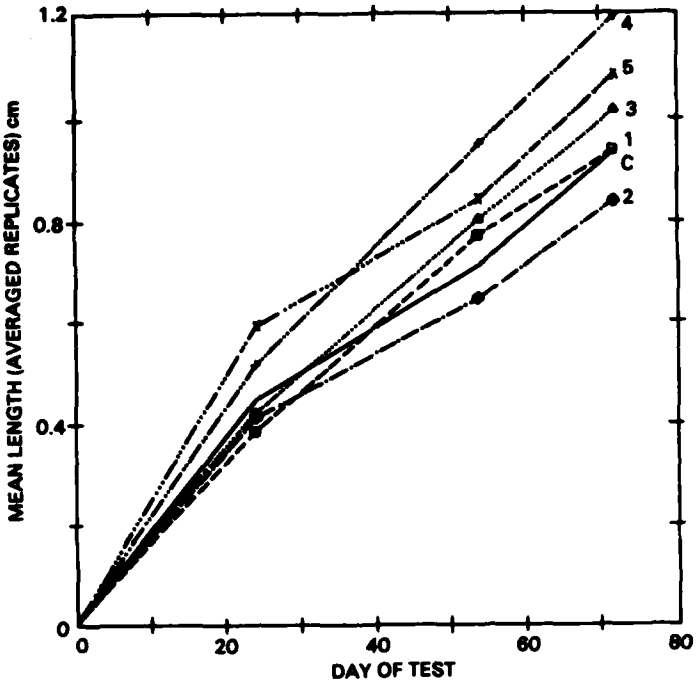

Figure 13. Flatfish length increase recorded during chronic toxicity testing.

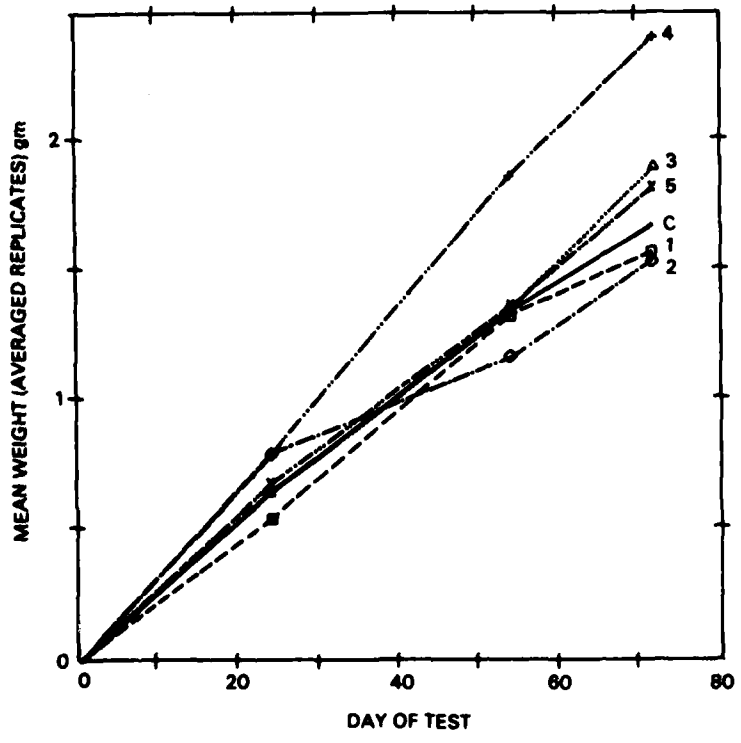

Figure 14. Flatfish whole body weight increase recorded during chronic toxicity testing.

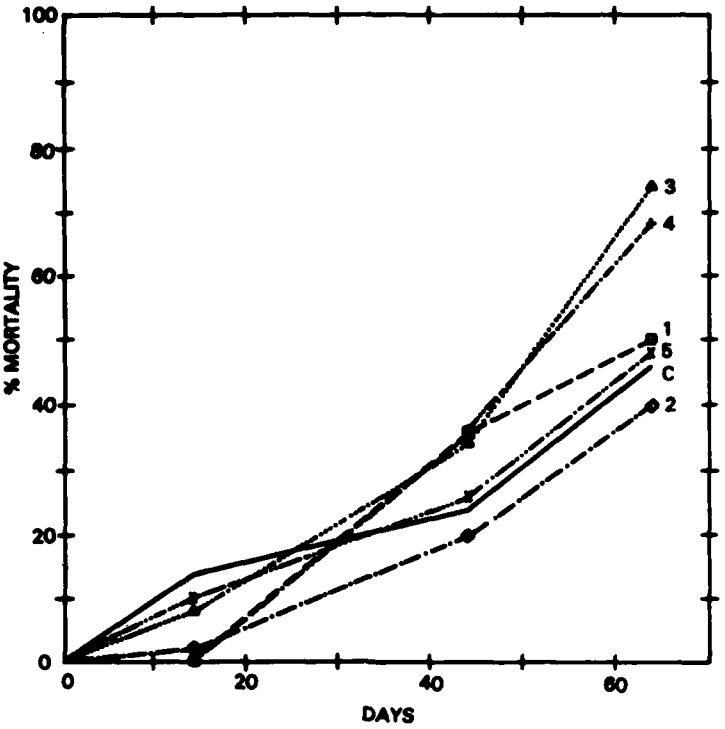

Figure 15. Flatfish cumulative mortality recorded during chronic toxicity testing.

Previously, an estimated 70-day LC_{50} of 2.8-ppb tributyltin was determined for flatfish exposed to leachates from OMP M253 antifouling paint (FY 82 progress report, unpublished data). This value was, however, an estimate based on mortality data observed at the highest tributyltin concentration (1.58 ppb) tested and, hence, an extrapolation. The results of the FY 82 study and this current investigation indicate a long-term LC_{50} for tributyltin may lie within the range of 1.89-2.80 ppb, or possibly slightly higher. These values are comparable to those reported for other fish species tested for long-term (> 96-hour) periods (Seinen et al., 1981; Ward et al., 1981).

2. Oysters

More than 90 percent of all oysters tested within each concentration survived tributyltin exposure from 0.04-1.89 ppb for the 67-day testing period. Control survival was also greater than 90 percent. Mortality in other adult oyster species has been reported by other investigators. Approximately 50 percent of oysters tested in bioaccumulation studies conducted with *Crassostrea gigas* and *Ostrea edulis* died within 21 days at a tributyltin concentration of 1.25 ppb (Waldock et al., 1983). Total mortality was recorded after 31 days of exposure at 1.25 ppb with *Crassostrea gigas* and after 44 days of exposure with *Ostrea edulis*. Field trials with *Crassostrea gigas* exposed to plates coated with tributyltin antifouling paint resulted in high oyster mortality (Alzieu et al., 1982). Laboratory studies with estimated tributyltin concentrations of 0.2 and 2.0 ppb led to 30-percent mortality after 110 days and total mortality after 50 days, respectively (Alzieu, 1981).

Our results demonstrated that *Crassostrea virginica* could tolerate a 67-day exposure to 1.89-ppb tributyltin. Possibly, the testing conditions with respect to salinity and temperature permitted some degree of resistance since they were somewhat different from the normal range experienced by this species. Nevertheless, survival was greater than 90 percent at 1.89 ppb, and abnormal shell growth was not observed at any concentration tested from 0.04-1.89 ppb. We are not aware of any other studies addressing tributyltin toxicity to *Crassostrea virginica*. This oyster species may represent a more resistant form than other oyster species with respect to tributyltin exposure.

3. Mussels

The cumulative percent mortality observed during the course of the chronic test is shown in Figure 16. A probit analysis (SAS User Guide, 1982) using mortality data from test tanks within a given concentration as replicates was subsequently conducted. The results of probit analysis conducted with mussel mortality data expressed as probability versus tributyltin concentration are shown in Figure 17. An LC_{50} value of 0.97-ppb tributyltin was calculated using untransformed mortality data from the five test concentrations over the 66-day testing period. Upper and lower 95-percent fiducial limits (limits estimated from the variability inherent in the data but not based on a normal distribution characteristic of confidence limits) of 1.65- and 0.62-ppb tributyltin were determined, respectively.

A concentration of 0.05-ppb tributyltin was toxic to 8 percent of the mussels tested over the 66-day period (Figure 17). This value approximates the lowest test concentration where mortality greater than that seen in the controls was observed (concentration 1=0.04 ppb) and may be considered near the toxic threshold limit for mussels tested in this study. Fiducial limits (95 percent) were, however, quite broad ranging from 0 to 0.42-ppb tributyltin at this lower test concentration. To better define the tributyltin toxic threshold to mussels, a more definitive study with test concentrations bracketing 0.05 ppb is advisable.

Ninety-percent mortality has been observed in static bioassay tests with *Mytilus edulis* for a 96-hour period at 76-ppb tributyltin (Workshop on Environmental Impact, 1983). Acute mortality at 38.0 µg/liter tributyltin has recently been reported for *Mytilus edulis* over a 96-hour testing period (Thain, 1983). The long-term LC_{50} of 0.97-ppb tributyltin determined in our study is 2.6 percent of this acute value, emphasizing the importance of long-term toxicity testing with compounds which may be slow-acting in initiating toxicity in test species. Recently, an LC_{50} value of 0.1 ppb for *Mytilus edulis* larvae over a 15-day exposure period has been reported (Beaumont & Budd, 1984), representing an acute to chronic toxicity ratio of 380 between adult and larval mussels. The view that short-term toxicity testing (96-hour) with tributyltin, which acts slowly in manifesting toxic effects, may seriously underestimate toxicity and set limits much higher than acceptable values is supported by other investigators (Laughlin et al., 1982). Toxicity to amphipods was apparent only after 5 days of testing with tributyltin concentrations ranging from 6 to 15 ppb (Laughlin et al., 1982). Clearly, 96-hour toxicity data in terms of LC_{50} estimations at such low concentrations are of little value. Long-term testing must be performed as well to determine toxicity to test species at environmentally realistic tributyltin concentrations.

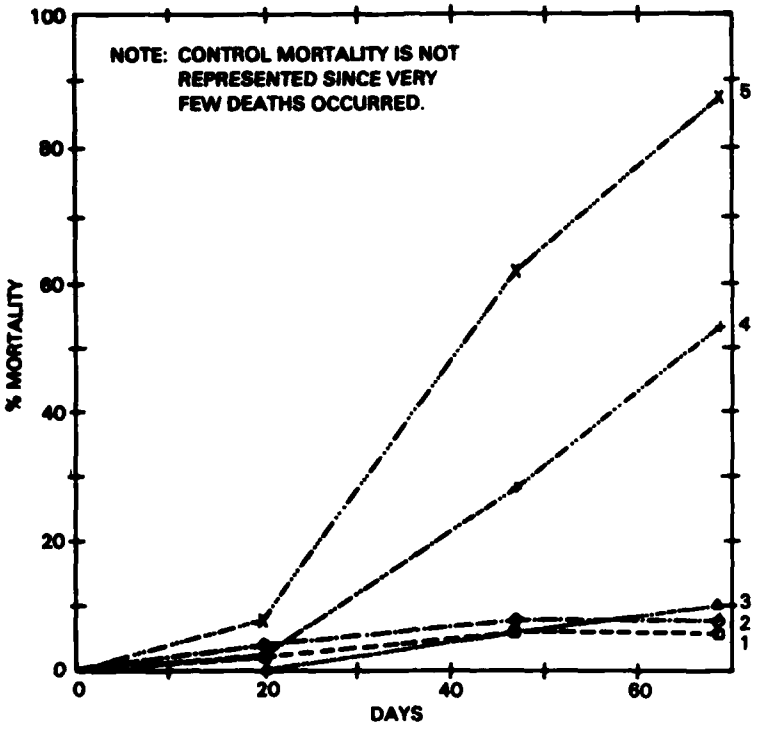

Figure 16. Mussel cumulative mortality recorded during chronic toxicity testing.

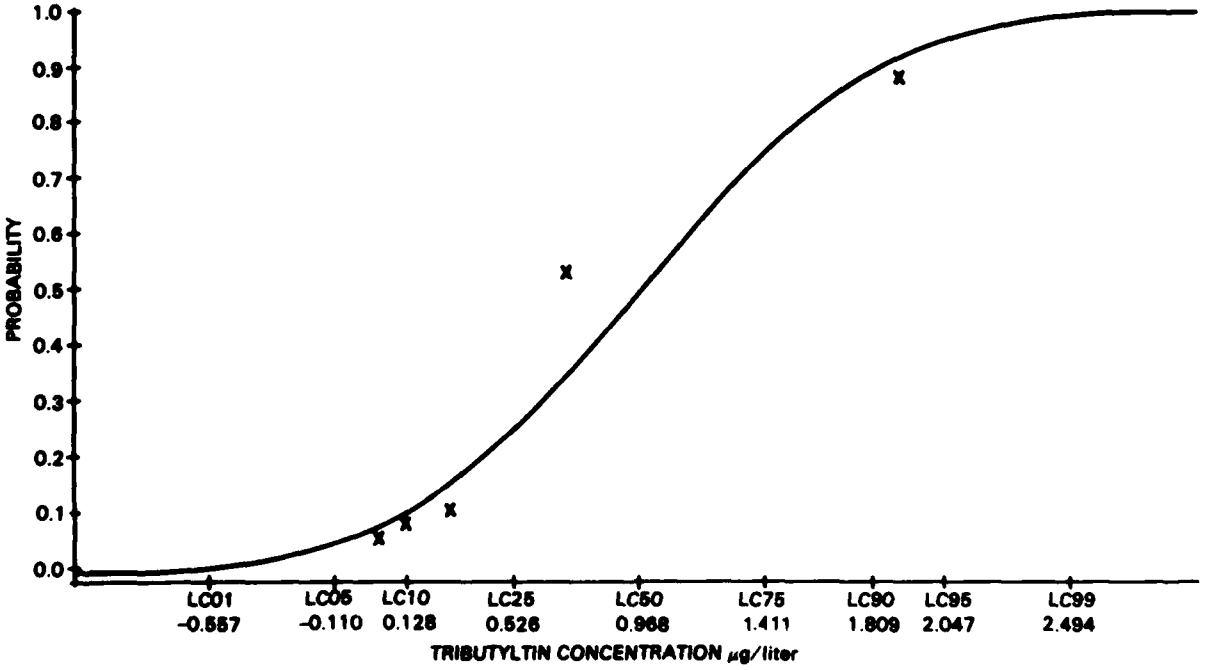

Figure 17. Mussel mortality probit analysis.

When exposed to tributyltin, mussels frequently exhibit an inability to normally close their valves. This failure to adequately close their valves accompanies a moribund state and is functionally important from an ecological view since a mussel in such a condition, while not yet dead, cannot protect itself from predators. During the course of the long-term toxicity test, an attempt was made to identify the tributyltin concentration and length of exposure necessary to cause this response. Mussels were checked daily and considered moribund if they failed to close their valves after 10 seconds of prodding with a glass rod. Normal closing was accomplished after 1-5 seconds.

A moribund response was noted in some mussels after the 19th day of exposure in test concentration 3 and in concentrations 5 and 4 after the 22nd and 35th days, respectively. The number of moribund individuals increased with exposure time, particularly in concentration 5 (Figure 18). The number of moribund mussels observed during the long-term toxicity test did not equal the number dead at a given period after the 20th day of exposure and, hence, did not appear to be a precondition to death since the cumulative mortality was always higher in a given concentration at a particular time period (Figures 16 and 18).

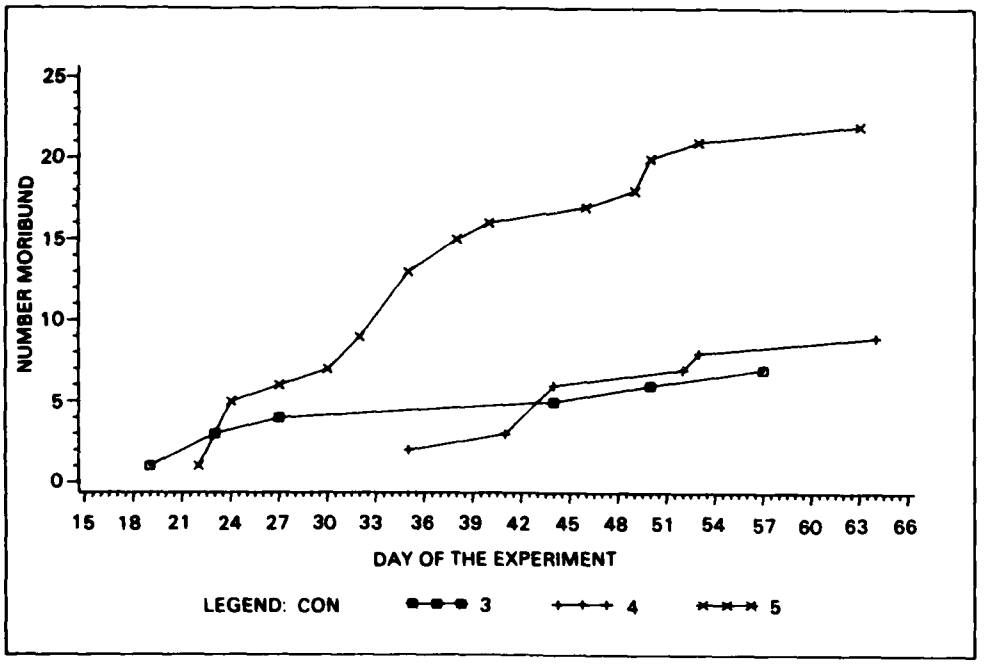

Figure 18. Cumulative moribund mussels observed during long-term exposure to tributyltin.

4. Mysids

Adult and juvenile mysids (*Acanthomysis sculpta*) were tested for 96-hour periods to determine acutely toxic tributyltin concentrations with this sensitive marine species. Acute 96-hour LC_{50} values of 0.61- and 1.68-ppb tributyltin were determined for juvenile and adult species, respectively. Control survival with juveniles was 96 percent of the initial number of specimens tested after 96 hours. Ninety-four percent of the adults

tested in control tanks survived after 96 hours. Long-term testing beyond the 96-hour period was not successful due to declining control survival with juvenile mysids, possibly as a result of inadequate food availability. Long-term toxicity tests with juvenile and adult *Acanthomysis sculpta* species will be performed in FY 85 to determine the relationship between acute and long-term toxicity with this species.

The current data indicate juvenile *Acanthomysis sculpta* were more sensitive to tributyltin than were adult specimens by a factor of nearly three. Toxicity data (96-hour LC_{50} of 1.68 ppb) for adult *Acanthomysis sculpta* are similar, but lower than 96-hour LC_{50} data (3.3 ppb) determined for another mysid species, *Metamysidopsis elongata* (NOSC FY 83 progress report, unpublished data).

The toxicity data determined for both mysid species and juvenile *Acanthomysis sculpta* are similar to LC_{50} values reported for other sensitive marine crustacea. Total mortality was noted with the amphipod species *Gammarus oceanicus* after 5 days of testing at 4.8-ppb tributyltin introduced in seawater solution as a leachate from antifouling paint (Laughlin et al., 1982). A 96-hour LC_{50} value of 1.0-ppb tributyltin was determined for the marine copepod (*Acartia tonsa*) in toxicity tests performed at NOSC (U'Ren, 1983). Toxicity was apparent in some *Acartia tonsa* species, which became moribund after 6 days of exposure to 0.3-ppb tributyltin. This latter value is representative of tributyltin concentrations measured in yacht harbors within San Diego Bay (NOSC Baseline Survey Data, unpublished).

Recently, long-term effects of tributyltin on the amphipod species *Gammarus oceanicus* have been reported (Laughlin et al., 1984). Larval *Gammarus oceanicus* were exposed to TBTO and tributyltin fluoride (TBTF) for 8 weeks. No larval *Gammarus oceanicus* survived at 3.0-ppb TBTO or TBTF (Laughlin et al., 1984). These results as well as the current data indicate realistic toxic tributyltin concentrations to marine crustacea, such as amphipods, copepods, and mysids, are within the range of 0.3-3.0 ppb or possibly less, depending on the species and developmental stage.

A recent report has documented tributyltin toxicity (LC_{50}) to larval bay mussels (*Mytilus edulis*) at 0.1 ppb after a 15-day exposure period (Beaumont & Budd, 1984). This study reports the lowest tributyltin concentration at which significant toxicity to test species has been observed to our knowledge. Continued testing with long-term exposure to tributyltin may confirm and identify other marine species sensitive to tributyltin at sub-ppb concentrations.

SUMMARY

SUBLETHAL GROWTH

1. Mussels

No significant changes in shell widths or weights of whole specimens were noted after long-term exposure to tributyltin at concentrations ranging from 0.04-1.89 ppb. A significant difference in shell length was observed at the end of the 65-day testing period. Shell lengths in the control treatment were significantly different from those in concentrations 3 and 4 ($p < 0.05$). A decrease in shell length was evident in treatments where specimen survival was 50 percent or better; hence, a more balanced statistical treatment was possible. The decrease in shell length with increasing tributyltin concentration followed the order: control, 1, 2, 3, and 4.

Condition indices calculated for mussels surviving the long-term toxicity test were not significantly different among the control treatment and test concentrations. No effect on mussel condition indices was observed with increasing tributyltin concentrations.

2. Oysters

Shell growth was minor in terms of increases in length and width. Condition indices calculated for surviving oysters in the control treatment and test concentrations 1, 2, and 3 were found to be significantly larger than those calculated fo oysters exposed to 0.73- and 1.89-ppb tributyltin in concentrations 4 and 5, respectively. The decrease in condition index with increasing tributyltin concentration has recently been observed by other investigators as well.

3. Fish

No significant differences in length or weight were noted in specimens among the tributyltin concentrations tested. Growth in terms of length and weight was positive throughout the testing period in test concentrations and in controls indicating sublethal growth was not adversely affected at the tributyltin concentrations tested (0.04-1.89 ppb).

MORTALITY

1. Fish

Mortality was not associated with increasing tributyltin concentrations during the 65-day testing period. Specimens in the control and highest test concentration exhibited similar cumulative mortality percentages of 47-48 percent. The high mortality percentage observed in control specimens was indicative of potential problems with experimental conditions, possibly associated with excess waste presence in test containers. The data presented in this study and mortality estimates previously determined at NOSC (FY 82 year-end report) suggest a long-term LC_{50} value for *Citharichthys stigmaeus* may lie within the range of 1.89-2.80 ppb or slightly higher.

2. Oysters

More than 90 percent of *all* oysters tested within each concentration survived the long-term test at tributyltin concentrations ranging from 0.04 to 1.89 ppb. This species exhibited a greater resistance to tributyltin exposure than previously reported in the literature for other oyster species. Mortality data for other oyster species have been reported at tributyltin concentrations of 2 ppb or less.

3. Mussels

An LC_{50} value of 0.97-ppb tributyltin (95-percent fiducial limits of 1.65-0.62 ppb) was determined for *Mytilus edulis* after the 66-day testing period. This value is 2.6 percent of acute 96-hour LC_{50} data reported in the literature for this species. Ninety-percent mortality was observed with *Mytilus edulis* tested under static bioassay conditions for a 96-hour period at 76-ppb tributyltin. The large difference in LC_{50} data between long-term and acute 96-hour toxicity tests emphasizes the importance of long-term bioassay testing for assessment of realistic environmental toxicity levels, particularly with slow-acting toxicants such as tributyltin.

4. Mysids

An acute 96-hour LC_{50} value of 0.61-ppb tributyltin was determined for juvenile *Acanthomysis sculpta*. Adult specimens exhibited an acute 96-hour LC_{50} value of 1.68 ppb, indicating juvenile specimens were more sensitive to tributyltin than adults by nearly a factor of 3. Species-dependent toxicity was also apparent. The 96-hour LC_{50} value of 1.68 ppb determined for *Acanthomysis sculpta* in this study was less than the 96-hour LC_{50} value of 3.3 ppb determined for another mysid species, *Metamysidopsis elongata*, tested previously at NOSC. The toxicity data determined for both mysid species are similar to values reported for other marine crustacea. A 96-hour LC_{50} value of 1.0-ppb tributyltin has been reported for a copepod species. Adult amphipods have exhibited 50-percent mortality at 3.0 ppb (TBTO and TBTF) after 10-12 days of exposure. A significant reduction ($p = 0.0012$) in the number of larval amphipods produced from adults exposed to 0.3 µg/liter TBTO or TBTF for 8 weeks was also reported (Laughlin et al., 1984).

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ABBREVIATIONS

ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
cc	Cubic centimeter
cm	Centimeter
CV	Coefficient of variation
FY	Fiscal year
gm	Gram
LC_{50}	Test concentration where 50-percent mortality is observed for a given time period
µg	Microgram
ml	Milliliter
mm	Millimeter
MRT	Multiple range test
NOSC	Naval Ocean Systems Center
OMP	Organometallic polymer
p	Statistical level of significance (alpha)
ppb	Parts per billion
ppt	Parts per thousand
PVC	Polyvinyl chloride
SPC	Self-polishing copolymer
STDV	Standard deviation
TBTCL	Tributyltin chloride
TBTF	Tributyltin fluoride
TBTO	Bis (tri-n-butyltin) oxide
Vac	Volts alternating current
$\bar{x}$	Mean of "n" numbers

APPENDIX A

WATER QUALITY DATA

Legend:

1. C = chronic test
   B = biochemical test
   A = bioaccumulation test

2. O = oyster
   M = mussel
   F = fish

3. First numeric column = test concentration 0, 1, 2, 3, 4 or 5

   Second numeric column = replicate tank number 1, 2, 3, 4 or 5

19 APR 84

Day	Sample No.	Temp.	O_2	pH
3	C041	17.0	8.7	7.97
3	C042	17.0	8.9	8.00
3	C043	17.0	8.7	8.01
3	C044	17.0	8.8	8.02
3	C045	17.0	8.8	8.03
3	B041	17.0	8.7	8.00
3	B042	17.0	8.7	8.01
3	B043	17.0	8.8	8.00
3	B044	17.0	8.8	8.01
3	A043	17.0	8.8	8.05
3	CF41	17.0	8.4	8.02
3	CF42	17.0	8.4	8.00
3	CF43	17.0	8.8	8.01
3	CF44	17.0	8.6	8.01
3	CF45	17.0	8.8	8.01
3	AF41	17.5	8.5	7.97
3	AF42	17.5	8.2	7.96
3	AF43	17.0	8.4	7.98
3	A041	17.0	8.8	8.04
3	A042	17.0	8.8	8.05
3	CM41	17.0	8.7	8.08
3	CM42	17.0	8.7	8.08
3	CM43	17.0	8.8	8.08
3	CM44	17.0	8.7	8.09
3	CM45	17.0	8.8	8.08
3	BM42	17.0	8.6	8.06
3	BM41	17.0	8.7	8.05
3	BM43	17.0	8.7	8.05
3	BM44	17.0	8.7	8.06
3	A044	17.0	8.6	8.06
3	C011	17.5	8.9	7.96
3	C012	17.5	9.0	7.96
3	C013	17.5	9.0	7.96
3	C014	17.5	9.0	7.96
3	C015	17.5	9.0	7.96
3	A011	17.5	9.0	7.95
3	A012	17.5	9.0	7.94
3	A013	17.5	9.0	7.94
3	A014	17.5	9.1	7.95
3	A015	17.5	9.1	7.95
3	CF11	17.0	8.8	7.94
3	CF12	17.0	8.7	7.94
3	CF13	17.5	8.8	7.94
3	CF14	17.5	8.6	7.93
3	CF15	17.5	8.6	7.92
3	AF11	17.5	8.0	7.82
3	AF12	17.5	8.7	7.85
3	AF13	17.5	8.2	7.84
3	AF14	17.5	8.1	7.83
3	AF15	17.0	8.2	7.83

19 APR 84 (Continued)

Day	Sample No.	Temp.	O_2	pH
3	CM11	17.5	9.0	7.94
3	CM12	17.0	9.0	7.96
3	CM13	17.0	8.9	7.96
3	CM14	17.0	8.9	7.96
3	CM15	17.0	8.8	7.96
3	AM11	17.0	8.9	7.95
3	AM12	17.0	8.8	7.96
3	AM13	17.0	8.9	7.96
3	AM14	17.0	8.8	7.96
3	AM15	17.0	8.9	7.96
3	C021	17.5	9.0	7.98
3	C022	17.5	9.4	7.98
3	C023	17.5	9.0	7.98
3	C024	17.5	9.0	7.97
3	C025	17.5	9.0	7.97
3	B021	17.5	8.9	7.96
3	B022	17.5	9.0	7.97
3	B023	17.5	9.0	7.96
3	B024	17.5	8.9	7.96
3	A021	17.5	9.0	7.97
3	CF21	18.0	8.6	7.94
3	CF22	18.0	8.6	7.93
3	CF23	18.0	8.5	7.94
3	CF24	18.0	8.7	7.95
3	CF25	18.0	8.9	7.95
3	AF21	18.0	9.0	7.97
3	AF23	17.5	9.1	7.98
3	AF22	17.5	9.2	7.98
3	A023	17.5	9.0	7.96
3	CM21	17.5	9.0	7.98
3	CM22	17.5	9.1	7.98
3	CM23	17.5	9.1	7.98
3	BM22	17.5	9.0	7.96
3	CM24	17.5	9.0	7.97
3	CM25	17.5	9.0	7.97
3	BM21	17.5	8.9	7.97
3	BM23	17.5	8.9	7.96
3	BM24	17.5	8.9	7.97
3	A024	17.5	9.1	7.95
3	A022	17.5	9.1	7.96
3	C031	17.0	8.6	8.15
3	C032	17.0	8.8	8.16
3	C033	17.0	8.8	8.16
3	C034	17.0	8.7	8.16
3	C035	17.0	8.8	8.15
3	A031	17.0	8.7	8.15
3	A032	17.0	8.7	8.14
3	A033	17.0	8.7	8.14
3	A034	17.0	8.7	8.13
3	A035	17.0	8.7	8.13

19 APR 84 (Continued)

Day	Sample No.	Temp.	O_2	pH
3	CF31	17.0	8.4	8.11
3	CF32	17.0	8.6	8.11
3	CF33	17.0	8.5	8.12
3	CF34	17.5	8.6	8.12
3	CF35	17.5	8.5	8.11
3	AF31	17.5	7.9	8.00
3	AF32	17.5	7.9	7.99
3	AF33	17.0	7.6	7.97
3	AF34	17.0	7.6	7.96
3	AF35	17.5	7.4	7.98
3	CM31	17.0	8.7	8.15
3	CM32	17.0	8.7	8.16
3	CM33	17.0	8.7	8.16
3	CM34	17.0	8.7	8.16
3	CM35	17.0	8.7	8.16
3	AM31	17.0	8.7	8.15
3	AM32	17.0	8.7	8.15
3	AM33	17.0	8.6	8.15
3	AM34	17.5	8.7	8.16
3	AM35	17.5	8.6	8.16
3	CM01	17.0	9.0	7.98
3	CM02	17.0	9.0	7.98
3	CM03	17.0	8.9	7.98
3	CM04	17.0	9.0	7.98
3	CM05	17.5	9.0	7.98
3	BM01	17.5	8.9	7.96
3	BM02	17.0	9.0	7.95
3	BM03	17.0	8.8	7.95
3	BM04	17.5	8.9	7.95
3	AM01	17.5	9.0	7.96
3	AM02	17.5	9.0	7.96
3	AM03	17.5	8.9	7.97
3	AM04	17.5	9.0	7.97
3	AM05	17.5	9.0	7.97
3	CF01	17.0	8.6	7.95
3	CF02	17.5	8.6	7.93
3	CF03	17.0	8.7	7.91
3	CF04	17.5	8.7	7.94
3	CF05	17.5	8.6	7.95
3	B001	17.5	8.8	7.96
3	B002	17.5	8.8	7.96
3	B003	17.5	8.7	7.96
3	B004	17.5	8.8	7.96
3	AF01	17.5	8.3	7.90
3	AF02	17.5	8.4	7.87
3	AF03	18.0	8.5	7.87
3	AF04	18.0	8.5	7.90
3	AF05	17.5	8.2	7.86
3	C001	17.0	9.0	7.97
3	C002	17.0	8.9	7.96

19 APR 84 (Continued)

Day	Sample No.	Temp.	O_2	pH
3	C003	17.0	9.0	7.97
3	C004	17.5	9.0	7.97
3	C005	17.5	8.9	7.98
3	A001	17.5	8.8	7.97
3	A002	17.0	8.9	7.98
3	A003	17.5	8.9	7.96
3	A004	17.5	9.0	7.96
3	A005	17.5	8.9	7.96
3	CM51	17.5	8.9	7.94
3	CM52	17.5	8.9	7.94
3	CM53	17.5	8.9	7.95
3	CM54	17.5	8.9	7.95
3	CM55	17.5	8.9	7.95
3	BM51	17.5	8.6	7.93
3	BM52	17.5	8.6	7.90
3	BM53	17.5	8.7	7.92
3	BM54	17.5	8.7	7.91
3	AM51	17.0	8.8	7.93
3	AM52	17.0	8.8	7.93
3	AM53	17.0	8.8	7.94
3	AM54	17.0	8.8	7.93
3	AM55	17.0	8.7	7.93
3	CF51	17.5	8.6	7.91
3	CF52	17.5	8.6	7.91
3	CF53	17.0	8.6	7.92
3	CF54	17.0	8.6	7.92
3	CF55	17.0	8.4	7.91
3	B051	17.5	8.8	7.91
3	B052	17.5	8.8	7.93
3	B053	17.5	8.8	7.93
3	B054	17.5	8.8	7.93
3	AF51	17.5	8.5	7.87
3	AF52	17.5	8.2	7.84
3	AF53	17.5	8.2	7.78
3	AF54	17.5	8.2	7.80
3	AF55	18.0	8.1	7.81
3	C051	17.0	8.8	7.94
3	C052	17.0	8.7	7.94
3	C053	17.0	8.8	7.95
3	C054	17.0	8.8	7.96
3	C055	17.0	8.8	7.96
3	A051	17.0	8.7	7.94
3	A052	17.0	8.7	7.93
3	A053	17.0	8.8	7.92
3	A054	17.0	8.8	7.91
3	A055	17.0	8.8	7.92
8	B022	15.5	9.5	8.06
8	B021	15.5	9.5	8.04
8	C025	15.5	9.5	8.08
8	C024	15.5	9.6	8.08

19 APR 84 (Continued)

Day	Sample No.	Temp.	O_2	pH
8	C023	15.5	9.6	8.08
8	C022	15.0	9.6	8.08
8	C021	15.5	9.5	8.08
8	A021	15.0	9.5	8.09
8	B023	15.0	9.5	8.08
8	B024	15.5	9.5	8.07
8	AF21	16.9	9.5	8.11
8	CF25	16.0	9.5	8.05
8	CF24	15.5	9.5	8.03
8	CF23	15.5	9.3	8.01
8	CF22	15.5	9.4	8.00
8	CF21	15.0	9.2	7.99
8	A023	15.5	9.6	8.09
8	A022	15.0	9.6	8.05
8	AF22	15.0	9.5	8.07
8	AF23	15.0	9.4	8.09
8	BM21	15.5	9.1	8.05
8	CM25	15.5	9.4	8.07
8	CM24	15.5	9.4	8.08
8	BM22	15.5	9.3	8.05
8	CM23	15.0	9.5	8.07
8	CM22	15.5	9.4	8.08
8	CM21	15.5	9.3	8.08
8	A024	15.5	9.2	8.03
8	BM23	15.0	9.2	8.04
8	BM24	15.0	9.2	8.06
8	CM51	16.0	9.3	8.08
8	CM52	15.5	9.3	8.08
8	CM53	15.5	9.3	8.08
8	CM54	15.5	9.4	8.08
8	CM55	15.5	9.4	8 09
8	BM51	15.5	9.3	8.06
8	BM52	15.5	9.2	8.04
8	BM53	15.5	9.2	8.03
8	BM54	15.5	9.2	8.02
8	AM55	15.5	9.2	8.04
8	AM54	15.5	9.4	8.06
8	AM53	15.5	9.4	8.07
8	AM52	15.5	9.4	8.06
8	AM51	15.5	9.4	8.06
8	AF51	16.0	9.0	7.97
8	AF52	16.0	9.0	7.92
8	AF53	15.5	8.7	7.83
8	AF54	16.0	8.9	7.92
8	AF55	16.0	8.7	7.87
8	B054	15.5	9.4	8.02
8	B053	15.5	9.2	8.01

24 APR 84

Day	Sample No.	Temp.	O_2	pH
8	A052	15.5	9.4	8.04
8	A051	15.5	9.4	8.05
8	C055	15.5	9.5	8.08
8	C054	15.5	9.4	8.08
8	C053	15.5	9.4	8.08
8	C052	15.5	9.5	8.08
8	B052	15.5	9.4	8.08
8	B051	15.5	9.4	8.08
8	CF55	15.5	8.7	8.01
8	CF54	15.5	8.7	8.00
8	CF53	15.5	9.2	8.02
8	CF52	15.5	8.9	8.01
8	CF51	15.5	9.5	8.07
8	A053	15.5	9.5	8.05
8	A054	15.5	9.4	8.04
8	A055	15.5	9.5	8.05

25 APR 84

Day	Sample No.	Temp.	O_2	pH
9	A012	16.9	9.2	8.02
9	A011	16.9	9.3	8.02
9	C015	16.9	9.3	8.04
9	C014	16.9	9.3	8.04
9	C013	16.9	9.3	8.04
9	C011	16.9	9.3	8.04
9	A013	16.9	9.3	8.03
9	A014	16.9	9.3	8.02
9	A015	16.9	9.2	8.02
9	AF15	17.0	8.7	7.98
9	AF14	17.0	8.7	7.96
9	AF13	17.0	8.6	7.95
9	CF11	17.0	9.1	8.01
9	CF12	17.0	9.1	8.01
9	CF13	17.0	9.1	8.01
9	CF14	17.0	9.1	8.01
9	CF15	17.0	9.1	8.01
9	AF11	17.0	8.6	7.95
9	AF12	17.0	8.5	7.94
9	AF13	17.0	9.1	8.02
9	AF14	17.0	9.1	8.03
9	AF15	17.0	9.1	8.03
9	AM12	16.9	9.1	8.03
9	AM11	16.8	9.1	8.04
9	CM15	16.8	9.2	8.04
9	CM14	16.8	9.2	8.04
9	CM13	16.8	9.2	8.04
9	CM12	16.8	9.2	8.05
9	CM11	16.8	9.2	8.05
9	C012	16.9	9.3	8.04
9	C041	16.8	9.3	8.01
9	C042	16.8	9.2	8.01
9	C043	16.8	9.3	8.02
9	C044	16.8	9.3	8.02
9	C045	16.8	9.3	8.02
9	B041	17.0	9.1	7.96
9	B042	16.9	9.0	7.94
9	B043	17.0	9.3	8.01
9	B044	17.0	9.2	8.01
9	A043	16.9	8.9	7.96
9	CF41	17.0	8.8	7.99
9	CF42	17.0	8.9	8.00
9	CF43	17.0	9.0	8.00
9	CF44	17.0	9.0	7.99
9	CF45	17.0	8.9	7.99
9	AF41	17.0	9.0	7.99
9	AF42	17.0	9.0	7.99
9	AF43	17.0	9.1	8.01
9	A041	17.0	9.3	8.03
9	A042	17.0	9.3	8.03

25 APR 84 (Continued)

Day	Sample No.	Temp.	O_2	pH
9	CM41	16.5	9.2	8.04
9	CM42	15.0	9.2	8.02
9	CM43	16.9	9.3	8.03
9	CM44	17.0	9.3	8.04
9	CM45	17.0	9.2	8.04
9	BM42	16.9	9.0	8.01
9	BM41	16.9	9.2	8.01
9	BM43	17.0	9.2	8.02
9	BM44	17.0	9.2	8.02
9	A044	17.0	9.2	8.01

26 APR 84

Day	Sample No.	Temp.	O_2	pH
10	CM01	16.5	9.2	8.12
10	CM02	16.5	9.5	8.13
10	CM03	16.5	9.4	8.13
10	CM04	16.5	9.4	8.13
10	CM05	16.5	9.4	8.13
10	BM01	16.5	9.3	8.12
10	BM02	16.5	9.2	8.12
10	BM03	16.0	9.2	8.11
10	BM04	16.0	9.2	8.11
10	BM05	16.5	9.3	8.12
10	AM05	16.5	9.3	8.12
10	AM04	16.5	9.3	8.12
10	AM03	16.5	9.4	8.12
10	AM02	16.5	9.2	8.12
10	AM01	16.5	9.3	8.12
10	AF01	16.5	8.7	8.06
10	AF02	16.5	8.8	8.04
10	AF03	16.5	8.8	8.04
10	AF04	16.5	9.0	8.06
10	AF05	16.5	8.6	8.04
10	B004	16.0	9.2	8.09
10	B003	16.0	9.2	8.09
10	B002	15.0	9.1	8.08
10	B001	16.0	9.2	8.10
10	CF05	16.5	9.1	8.10
10	CF04	16.5	9.0	8.10
10	CF02	16.5	9.0	8.09
10	CF03	16.5	9.1	8.11
10	CF01	16.5	9.0	8.08
10	C002	16.0	9.2	8.09
10	C003	16.0	9.3	8.11
10	C004	16.5	9.3	8.12
10	C005	16.5	9.2	8.12
10	A001	16.5	9.1	8.11
10	A002	16.5	9.4	8.12
10	A005	16.5	9.1	8.12
10	A004	16.5	9.0	8.11
10	A003	16.5	9.1	8.11
10	C001	16.5	9.3	8.11
10	C031	16.5	9.2	8.10
10	C032	16.5	9.3	8.11
10	C033	16.5	9.3	8.11
10	C034	16.5	9.4	8.11
10	C035	16.5	9.4	8.11
10	A031	16.0	9.2	8.06
10	A032	16.0	9.3	8.09
10	A035	16.0	9.2	8.09
10	A034	16.0	9.2	8.09
10	A033	16.5	9.2	8.09
10	AF33	16.5	8.0	8.02

26 APR 84 (Continued)

Day	Sample No.	Temp.	O_2	pH
10	AF34	16.0	8.2	8.01
10	AF35	16.5	8.5	8.04
10	AF32	16.0	8.2	7.93
10	AF31	16.0	8.6	8.01
10	CF35	16.5	9.1	8.07
10	CF34	16.0	9.1	8.08
10	CF33	16.0	9.0	8.09
10	CF32	16.0	9.0	8.10
10	CF31	16.0	9.0	8.08
10	CM32	16.5	9.3	8.11
10	CM33	16.0	9.2	8.11
10	CM34	16.0	9.2	8.10
10	CM35	16.0	9.3	8.11
10	AM31	16.0	9.2	8.10
10	AM32	16.0	9.2	8.10
10	AM35	16.5	9.2	8.11
10	AM34	16.0	9.2	8.11
10	AM33	16.0	9.2	8.11
10	CM31	16.5	9.3	8.11

8 MAY 84

Day	Sample No.	Temp.	O_2	pH
22	CM01	17.5		7.98
22	CM02	17.5		7.97
22	CM03	17.5		7.97
22	CM04	17.5		7.98
22	CM05	17.5		7.98
22	BM01	17.5		7.97
22	BM02	17.5		7.97
22	BM03	17.5		7.95
22	BM04	17.5		7.94
22	AM05	17.5		7.96
22	AM04	17.5		7.97
22	AM03	17.5		7.97
22	AM02	17.5		7.97
22	AM01	17.5		7.98
22	AF01	17.5		7.93
22	AF02	17.5		7.92
22	AF03	17.5		7.92
22	AF04	17.5		7.93
22	B044	17.5		7.94
22	B043	17.5		7.94
22	B002	17.5		7.95
22	B001	17.5		7.96
22	CF05	17.5		7.93
22	CF04	17.5		7.95
22	CF02	17.5		7.95
22	CF03	17.5		7.97
22	CF01	17.5		7.95
22	C001	17.5		7.96
22	C002	17.5		7.96
22	C003	18.0		7.96
22	C004	17.5		7.97
22	C005	17.0		7.98
22	A001	17.5		7.97
22	A002	17.5		7.96
22	A005	17.5		7.96
22	A004	17.5		7.97
22	A003	17.5		7.96
22	CM51	17.0		7.90
22	CM52	17.0		7.90
22	CM53	17.5		7.91
22	CM54	17.5		7.91
22	CM55	17.5		7.92
22	BM51	17.5		7.92
22	BM52	17.5		7.91
22	BM53	17.5		7.91
22	BM54	17.5		7.90
22	AM55	17.0		7.91
22	AM54	17.0		7.93
22	AM53	17.0		7.94
22	AM52	17.0		7.94

8 MAY 84 (Continued)

Day	Sample No.	Temp.	O_2	pH
22	AM51	17.0		7.95
22	AF51	17.5		7.88
22	AF52	17.5		7.90
22	AF53	17.5		7.89
22	AF54	17.5		7.89
22	AF55	17.5		7.88
22	B054	17.5		7.93
22	B053	17.5		7.93
22	B052	17.5		7.96
22	B051	17.5		7.96
22	CF55	17.5		7.92
22	CF54	17.5		7.94
22	CF53	17.5		7.94
22	CF52	17.5		7.92
22	CF51	17.5		7.93
22	A053	17.5		7.95
22	A054	17.0		7.97
22	A055	17.0		7.97
22	A052	17.5		7.97
22	A051	17.5		7.97
22	C055	17.5		7.97
22	C054	17.0		7.96
22	C053	17.0		7.97
22	C052	17.5		7.97
22	C051	17.5		7.98

10 MAY 84

Day	Sample No.	Temp.	O_2	pH
24	A015	18.0	7.4	7.92
24	A014	18.0	7.5	7.92
24	A013	18.0	7.4	7.92
24	C011	18.0	7.3	7.92
24	C012	18.0	7.3	7.92
24	C013	18.0	7.4	7.92
24	C014	18.0	7.4	7.92
24	C015	18.0	7.4	7.92
24	A011	18.0	7.4	7.92
24	A012	18.0	7.3	7.92
24	AF12	18.0	6.9	7.84
24	AF11	18.0	6.9	7.84
24	CF15	18.0	7.2	7.89
24	CF14	18.0	7.1	7.89
24	CF13	18.0	7.2	7.90
24	CF12	18.0	6.9	7.90
24	CF11	18.0	7.1	7.88
24	AF13	18.0	6.7	7.86
24	AF14	18.0	7.0	7.86
24	AF15	18.0	6.9	7.86
24	CM11	18.0	7.3	7.91
24	CM12	18.0	7.2	7.92
24	CM13	18.0	7.2	6.92
24	CM14	18.0	7.3	7.93
24	CM15	18.0	7.3	7.94
24	AM11	18.0	7.3	7.94
24	AM12	18.0	7.1	7.92
24	AM15	18.0	7.2	7.92
24	AM14	18.0	7.2	7.93
24	AM13	18.0	7.2	7.93
24	C041	17.5	7.3	7.91
24	C042	18.0	7.2	7.92
24	C043	18.0	7.2	7.92
24	C044	18.0	7.2	7.92
24	C045	18.0	7.2	7.93
24	B041	18.0	7.1	7.90
24	B042	18.0	7.1	7.90
24	B043	18.0	7.2	7.95
24	B044	18.0	7.3	7.95
24	A043	18.0	6.9	7.92
24	CF41	18.0	6.8	7.87
24	CF42	18.0	7.0	7.87
24	CF43	18.0	7.1	7.87
24	CF44	18.0	7.0	7.87
24	CF45	18.0	6.9	7.87
24	AF41	18.0	6.8	7.88
24	AF42	18.0	6.9	7.87
24	AF43	18.0	6.5	7.91
24	A041	18.0	6.8	7.88
24	A042	18.0	7.2	7.93

10 MAY 84 (Continued)

Day	Sample No.	Temp.	O_2	pH
24	CM41	18.0	7.0	7.91
24	CM42	18.0	7.0	7.91
24	CM43	18.0	7.1	7.91
24	CM44	18.0	7.0	7.91
24	CM45	18.0	7.1	7.91
24	BM42	18.0	6.8	7.89
24	BM41	18.0	6.9	7.89
24	BM43	18.0	7.1	7.91
24	BM44	18.0	7.1	7.92
24	A044	18.0	6.7	7.91

22 MAY 84

Day	Sample No.	Temp.	O_2	pH
36	CM01	20.0	7.3	7.99
36	CM02	20.0	7.3	8.01
36	CM03	18.5	7.3	8.01
36	CM04	19.5	7.2	8.02
36	CM05	19.5	7.2	8.03
36	BM01	20.0	7.3	8.03
36	BM02	19.5	7.2	8.04
36	BM03	19.5	7.0	8.01
36	BM04	19.5	7.1	8.01
36	AM05	19.5	7.1	8.04
36	AM04	19.5	7.1	8.03
36	AM03	19.5	7.1	8.04
36	AM02	19.5	7.2	8.03
36	AM01	19.5	7.2	8.04
36	AF01	19.5	6.8	7.98
36	AF02	19.5	6.9	7.95
36	AF03	19.5	7.0	7.98
36	AF04	20.0	7.0	7.98
36	AF05	19.5	6.6	7.94
36	B004	19.5	7.0	8.03
36	B003	19.5	7.2	8.03
36	B002	19.5	6.8	8.04
36	B001	19.5	6.8	8.04
36	CF05	19.5	6.5	8.00
36	CF04	19.5	7.2	8.02
36	CF02	19.5	7.2	8.01
36	CF03	20.0	7.3	8.05
36	CF01	19.0	7.1	8.03
36	C001	20.0	7.1	8.05
36	C002	19.5	7.1	8.06
36	C003	19.5	7.2	8.06
36	C004	19.5	7.2	8.06
36	C005	19.5	7.2	8.06
36	A001	19.5	7.2	8.06
36	A002	19.5	7.2	8.05
36	A005	19.0	7.1	8.05
36	A004	19.5	7.1	8.00
36	A003	20.0	7.2	8.05
36	C031	19.5	7.4	8.06
36	C032	19.5	7.4	8.06
36	C033	19.5	7.4	8.06
36	C034	19.5	7.4	8.06
36	C035	19.5	7.4	8.06
36	A031	19.5	7.3	8.06
36	A032	19.5	7.3	8.06
36	A035	19.0	7.2	8.06
36	A034	19.5	7.3	8.07
36	A033	19.5	7.2	8.06
36	AF33	19.5	6.3	7.97
36	AF34	19.5	6.3	7.96

22 MAY 84 (Continued)

Day	Sample No.	Temp.	O_2	pH
36	AF35	19.5	6.9	8.02
36	AF32	20.0	6.6	7.98
36	AF31	19.5	6.7	7.97
36	CF35	19.5	7.1	8.03
36	CF34	19.5	7.1	8.03
36	CF33	19.5	7.1	8.04
36	CF32	19.5	7.1	8.06
36	CF31	19.5	6.9	8.04
36	CM31	19.5	6.8	8.06
36	CM32	19.5	7.2	8.06
36	CM33	19.5	7.2	8.07
36	CM34	19.5	7.2	8.07
36	CM35	19.5	7.1	8.07
36	AM31	19.0	7.0	8.05
36	AM32	18.5	7.0	8.05
36	AM35	19.5	7.0	8.06
36	AM34	19.5	7.1	8.06
36	AM33	19.5	7.1	8.06

23 MAY 84

Day	Sample No.	Temp.	O_2	pH
37	A015	19.5	7.0	8.08
37	A014	19.5	7.1	8.08
37	A013	20.0	7.1	8.08
37	C011	19.5	7.0	8.09
37	C012	19.5	7.1	8.09
37	C013	20.0	7.1	8.09
37	C014	19.5	6.9	8.09
37	C015	20.0	7.0	8.09
37	A011	20.0	7.0	8.09
37	A012	19.5	6.9	8.08
37	AF12	19.5	6.5	7.96
37	AF11	19.5	6.4	7.97
37	CF15	20.0	6.8	8.04
37	CF14	20.0	6.8	8.05
37	CF13	20.0	7.0	7.07
37	CF12	19.5	6.7	8.01
37	CF11	19.5	6.8	8.04
37	AF13	19.5	6.6	8.02
37	AF14	19.5	6.8	8.02
37	AF15	19.5	6.8	8.02
37	CM11	19.5	6.9	8.06
37	CM12	19.5	6.9	8.07
37	CM13	19.5	7.0	8.08
37	CM14	20.0	7.1	8.08
37	CM15	20.0	7.0	8.08
37	AM11	20.0	7.0	8.08
37	AM12	20.0	7.0	8.08
37	AM15	19.5	6.9	8.07
37	AM14	20.0	6.9	8.07
37	AM13	19.5	6.8	8.07
37	C041	19.5	6.8	8.05
37	C042	19.5	6.8	8.04
37	C043	19.5	6.8	8.05
37	C044	19.5	6.8	8.05
37	C045	19.5	6.9	8.05
37	B041	19.5	6.8	8.03
37	B042	19.5	6.8	8.02
37	B043	19.5	7.0	8.05
37	B044	19.5	7.1	8.05
37	A043	19.5	6.9	8.03
37	CF41	19.5	7.0	8.04
37	CF42	19.5	7.0	8.04
37	CF43	19.5	7.1	8.03
37	CF44	19.5	7.0	8.03
37	CF45	19.5	7.1	8.03
37	AF41	19.5	7.2	8.04
37	AF42	19.5	7.2	8.05
37	AF43	19.5	7.2	8.06
37	A041	19.5	7.2	8.06
37	A042	19.5	7.3	8.07

23 MAY 84 (Continued)

Day	Sample No.	Temp.	O_2	pH
37	CM41	19.5	7.3	8.07
37	CM42	18.0	7.2	8.03
37	CM43	19.5	7.3	8.07
37	CM44	19.5	7.3	8.07
37	CM45	19.5	7.3	8.07
37	BM42	19.5	7.1	8.05
37	BM41	19.5	7.2	8.05
37	BM43	19.5	7.2	8.06
37	BM44	19.5	7.4	8.08
37	A044	19.5	7.4	8.09

24 MAY 84

Day	Sample No.	Temp.	O_2	pH
38	CM51	20.0	7.1	8.01
38	CM52	20.0	7.2	8.01
38	CM53	20.5	7.2	8.02
38	CM54	20.0	7.2	8.03
38	CM55	20.0	7.0	8.01
38	BM51	20.0	7.1	8.01
38	BM52	20.0	7.1	8.02
38	BM53	20.0	6.8	7.97
38	BM54	20.0	6.8	7.97
38	AM55	20.0	7.0	8.01
38	AM54	20.0	7.1	8.01
38	AM53	20.0	7.1	8.02
38	AM52	19.5	7.1	8.02
38	AM51	19.5	7.1	8.02
38	AF51	20.0	6.8	7.96
38	AF52	20.0	6.9	7.96
38	AF53	20.0	6.9	7.95
38	AF54	20.0	6.9	7.96
38	AF55	20.0	6.9	7.96
38	B054	20.0	6.9	7.99
38	B053	20.0	6.8	8.00
38	B052	19.5	7.0	8.02
38	B051	20.0	7.1	8.04
38	CF55	20.0	6.6	7.95
38	CF54	20.0	7.0	8.02
38	CF53	20.0	6.9	8.01
38	CF52	20.0	6.9	7.98
38	CF51	20.0	7.0	7.98
38	A053	19.5	7.0	8.03
38	A054	19.5	7.0	8.03
38	A055	19.0	6.9	8.03
38	A052	19.5	7.0	8.03
38	A051	19.5	7.0	8.03
38	C055	20.0	7.0	8.04
38	C054	20.0	7.1	8.04
38	C053	20.0	7.1	8.04
38	C052	20.0	6.9	8.05
38	C051	19.0	7.0	8.04
38	C021	20.0	7.2	7.95
38	C022	20.0	7.2	7.96
38	C023	20.5	7.3	7.97
38	C024	20.0	7.3	7.97
38	C025	20.0	7.3	7.97
38	B021	20.0	7.3	7.94
38	B022	20.0	7.2	7.95
38	B023	19.5	7.3	7.97
38	A021	20.0	7.3	7.99
38	CF21	20.0	7.3	7.96
38	CF22	20.0	7.3	7.93
38	CF23	20.0	7.3	7.93

24 MAY 84 (Continued)

Day	Sample No.	Temp.	O_2	pH
38	CF24	20.0	7.0	7.95
38	CF25	20.0	7.1	7.97
38	AF21	20.0	7.2	8.01
38	AF23	20.0	7.0	8.01
38	AF22	20.0	7.2	8.01
38	A022	19.5	7.1	7.99
38	A023	20.0	7.1	8.00
38	CM21	21.0	7.2	8.02
38	CM22	20.0	7.1	8.02
38	CM23	20.0	7.1	8.02
38	BM22	20.0	7.0	8.00
38	CM24	20.0	7.1	8.01
38	CM25	20.0	7.2	8.02
38	BM21	20.0	7.0	8.00
38	BM24	20.0	7.2	8.01
38	BM23	19.5	7.0	8.01
38	A024	20.0	7.1	7.98

APPENDIX B

DETERMINATION OF AMMONIA IN SEAWATER FROM BIOASSAY TEST SOLUTIONS

	Test Concentration	Replicate #	Ammonia as µg-at n/liter
Fish			
	Control	1	10.00
	1	2	10.00
	1	4	7.00
	2	1	20.00
	2	3	9.50
	3	1	1.20
	3	3	4.30
	4	2	12.00
	4	3	11.00
	5	5	19.00
Oysters			
	Control	1	0.35
	1	4	0.52
	3	1	1.00
	5	2	0.35
Mussels			
	Control	1	0.95
	2	2	1.90
	5	1	0.95
Seawater from reservoir tank			< .10

Detection limit = 0.1 µg/liter

APPENDIX C

COPPER ANALYSIS OF SEAWATER FROM BIOASSAY TEST CONCENTRATIONS

Treatment	Copper µg/liter
Control	7.5
1	5.0
2	7.5
3	5.0
4	7.5
5	10.0
Incoming seawater from reservoir	7.5

Conclusions:

1. Copper was not a significant co-toxicant present with tributyltin.

   A. Only treatment 5 leached copper at a concentration above that measured in ambient incoming seawater.

   B. The amount of copper leached in treatment 5 was less than twice the amount of tributyltin leached into the test solution (2.5 ppb vs. 1.89 ppb).

   C. Tributyltin is at least 10 times more toxic to copepods than copper (U'Ren, 1983); therefore, less than twice as much copper as tributyltin present in the highest test solution probably did not significantly contribute to the observed toxicity.

APPENDIX D

OYSTER GROWTH PARAMETERS

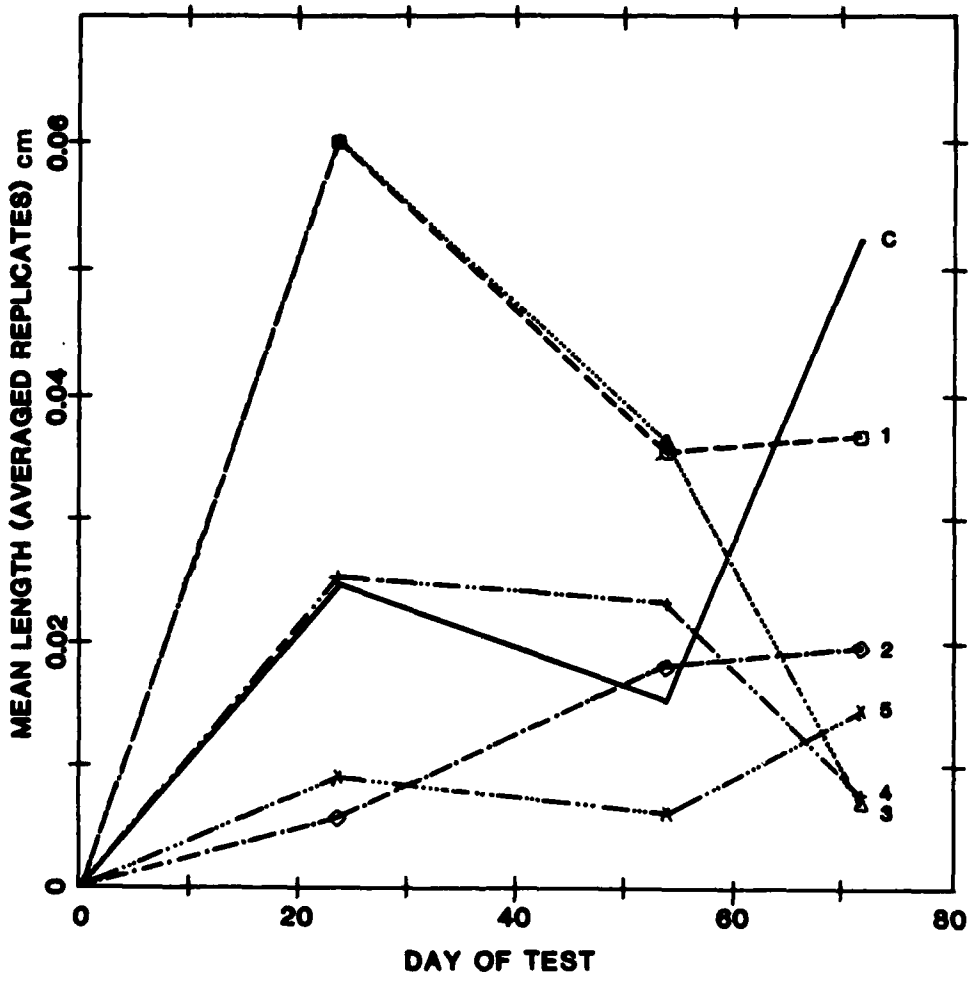

Figure D-1. Oyster growth (length).

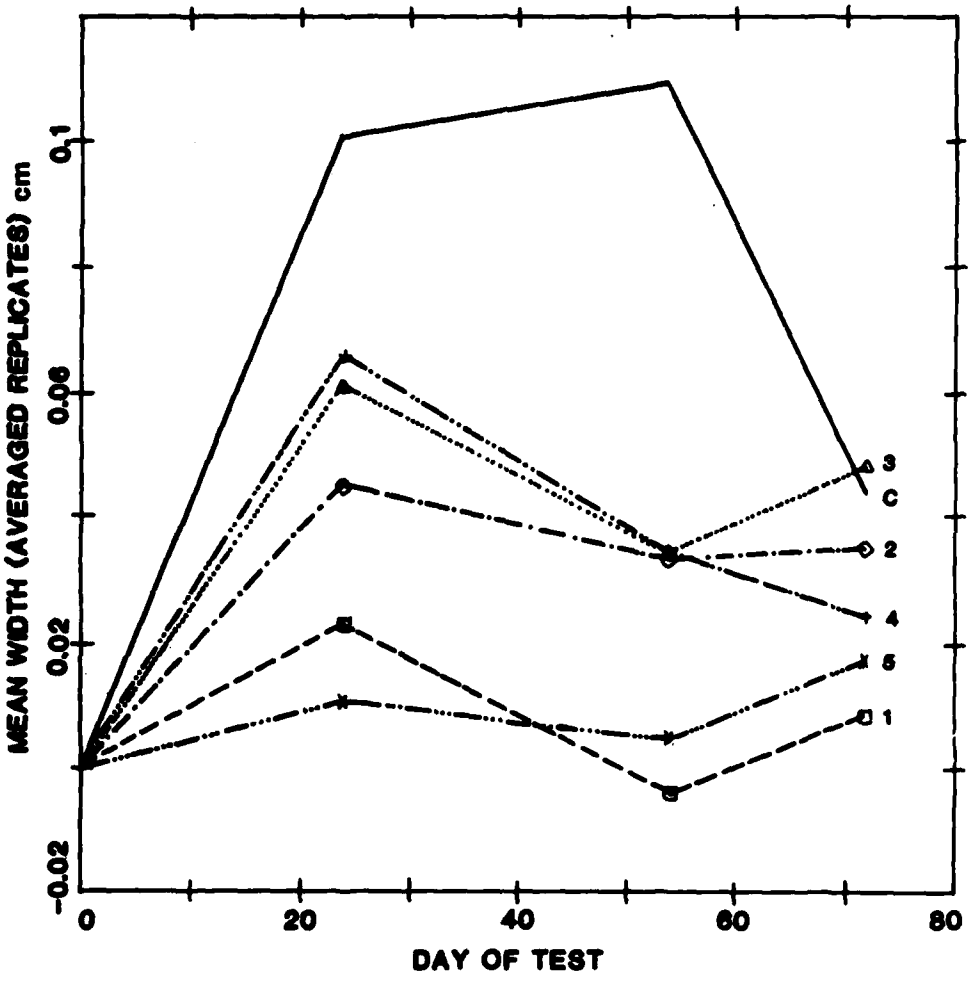

Figure D-2. Oyster growth (width).

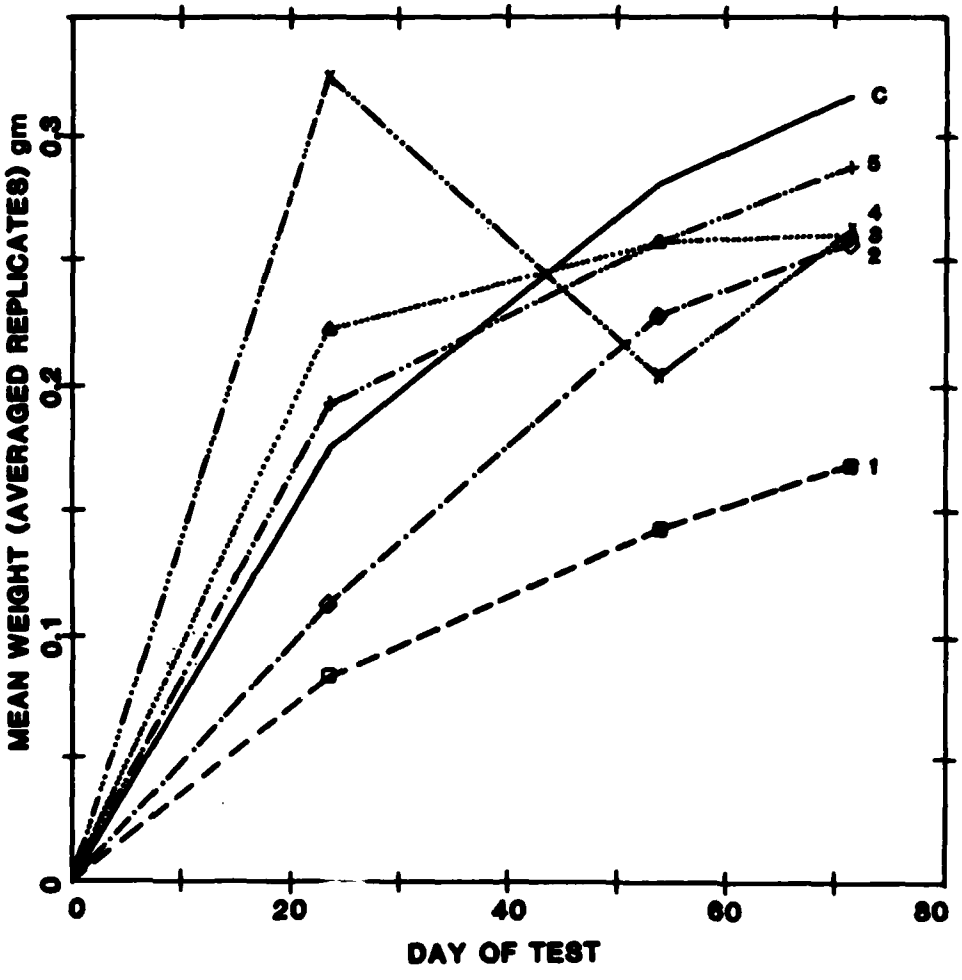

Figure D-3. Oyster growth (weight).

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